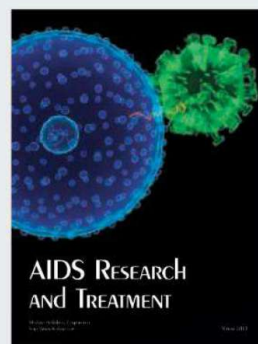
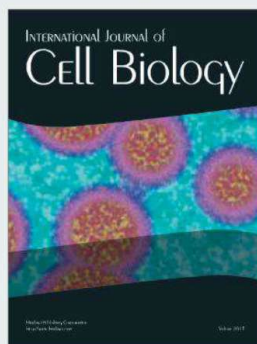
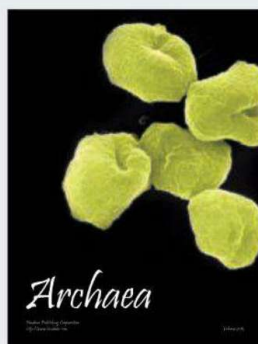
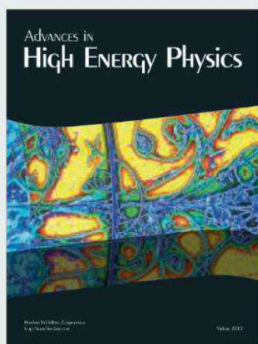
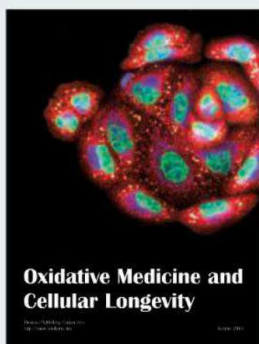
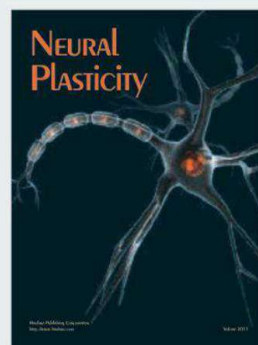
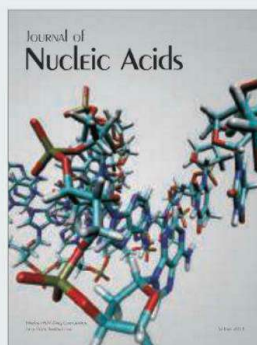
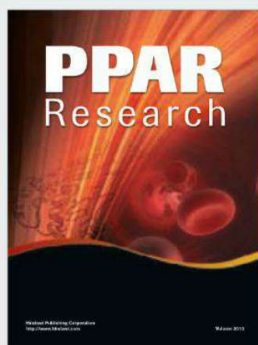
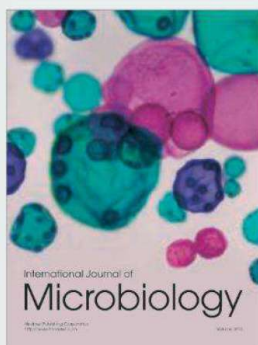
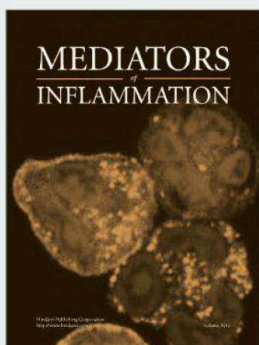
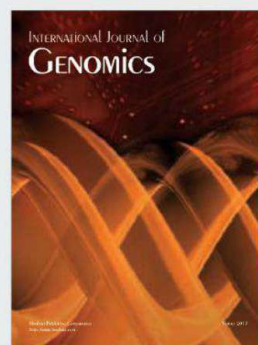
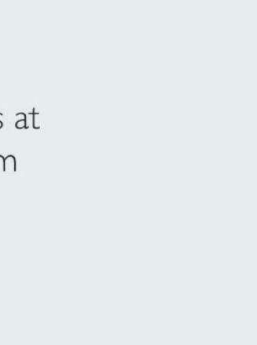
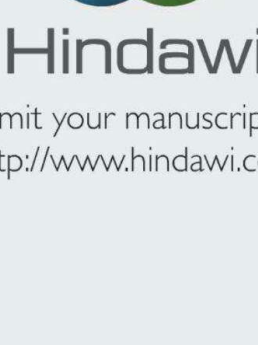
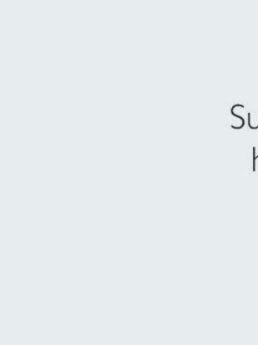
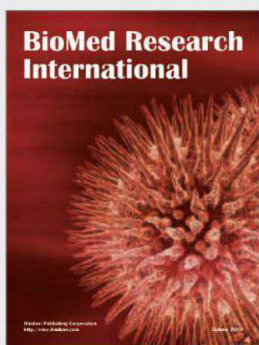
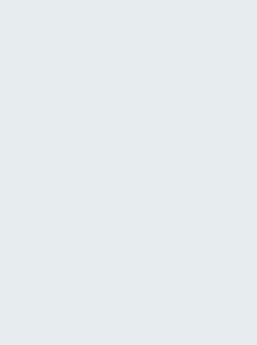
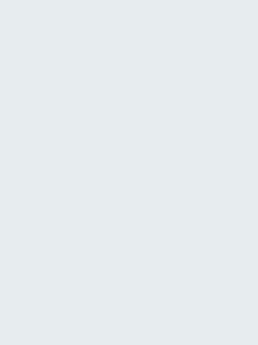
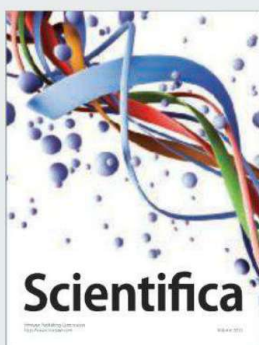
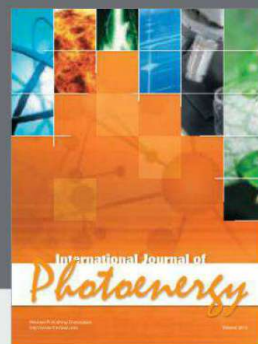
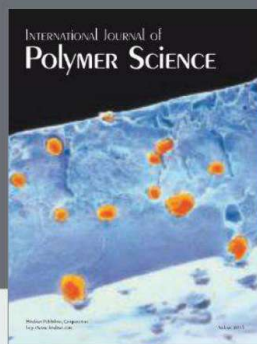
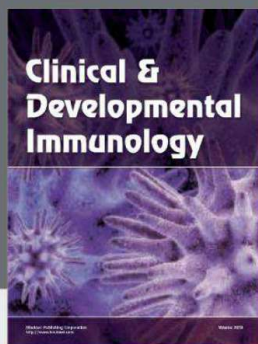
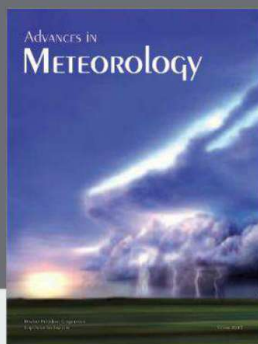
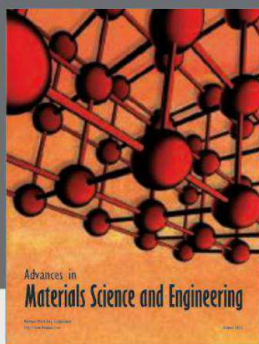


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Science

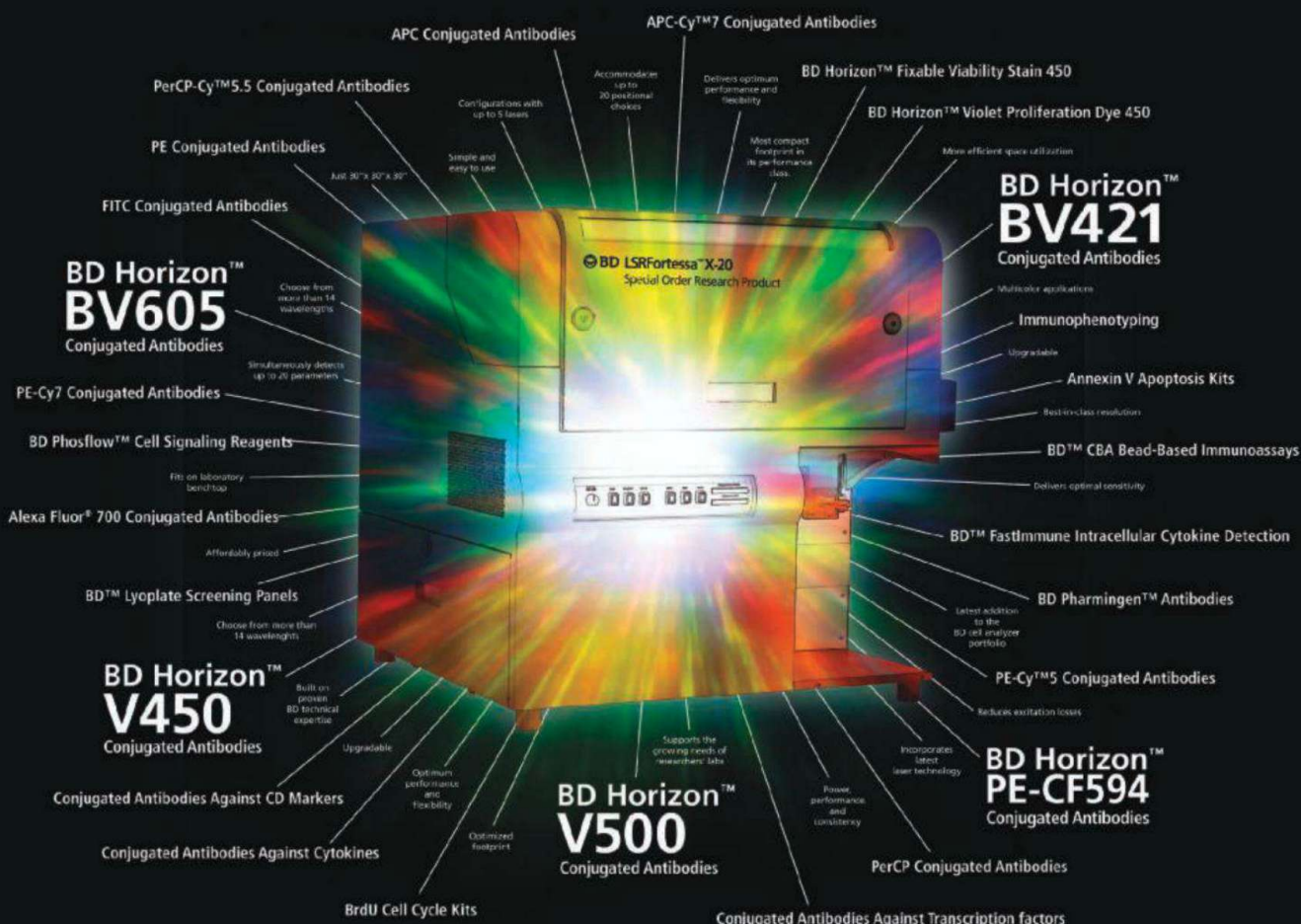


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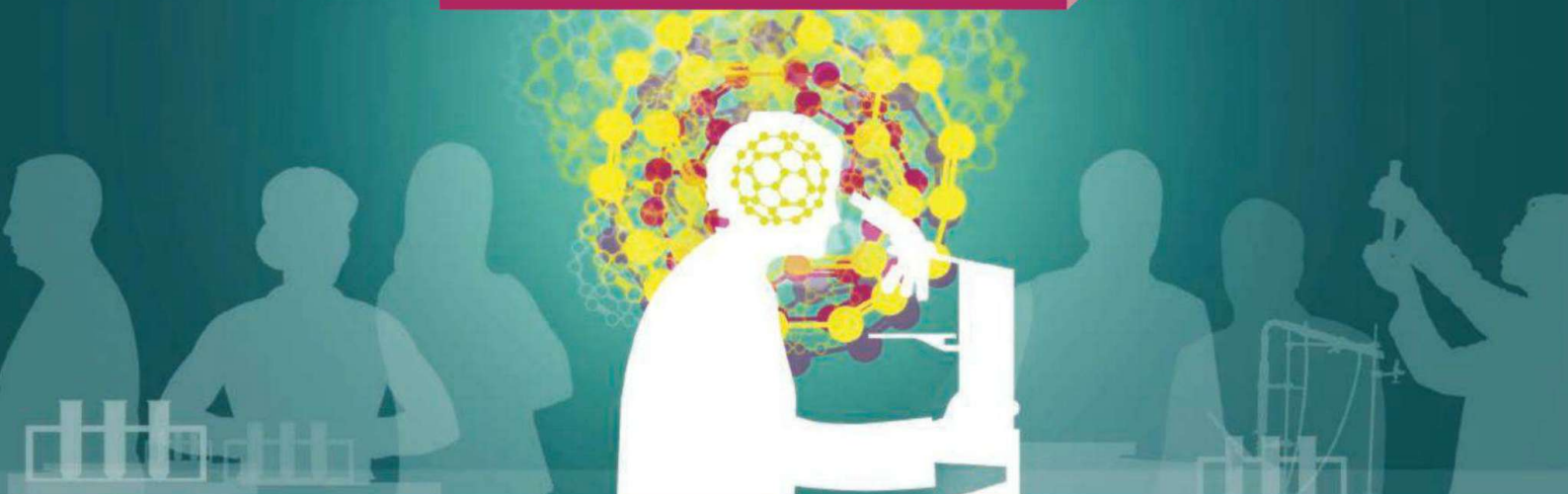
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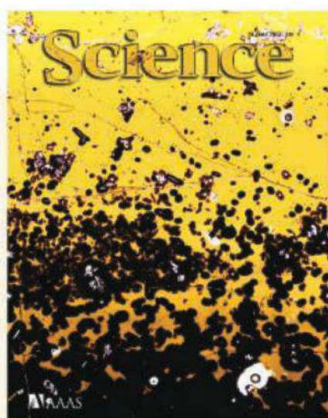
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ON THE WEB THIS WEEK

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Read about medieval versus modern leprosy, fossilized muscles for primitive jaws, ice shelf melting around Antarctica, and more.
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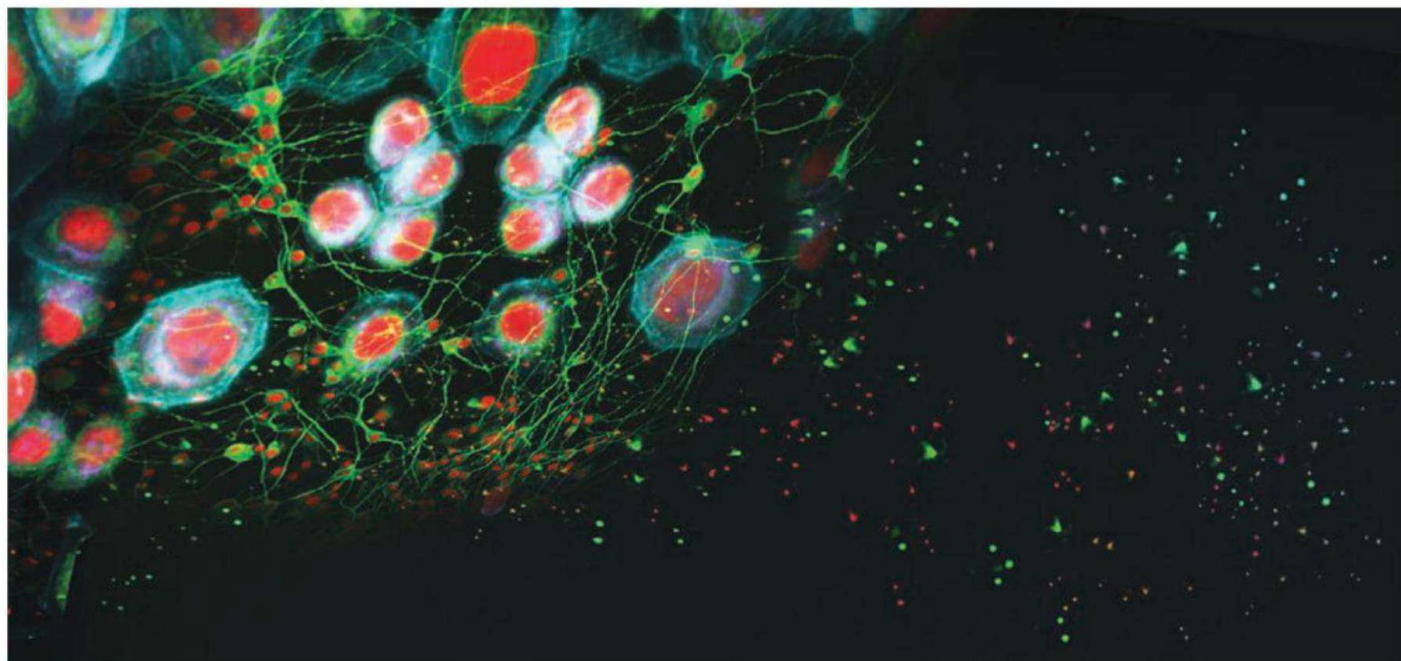
COVER

Polished thin section (70 micrometers) of volcanic glass, sample catalog number NMNH115296-3, in transmitted light (14 by 18 millimeters). Molten lava erupted onto the sea floor freezes to glass and minerals that contain clues to the lava's ancient past and origin in Earth's deep interior. Volcanic glasses such as this one may reveal a link between Earth's oxidation state and the deep carbon cycle. See page 1314.

Image: G. Macpherson, T. Gooding, and E. Cottrell

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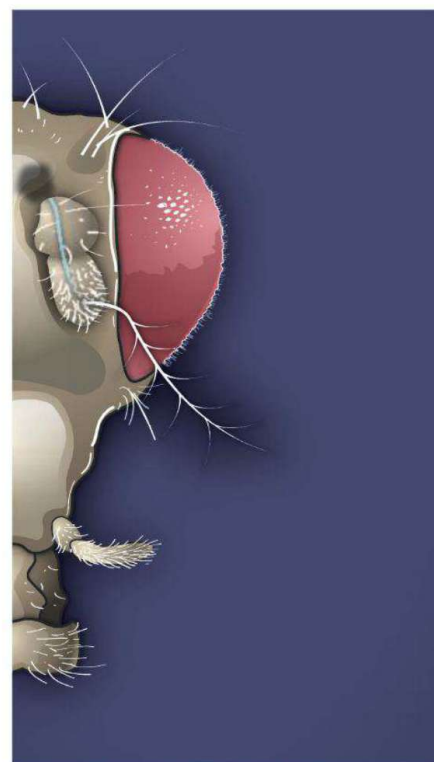
imagination at work

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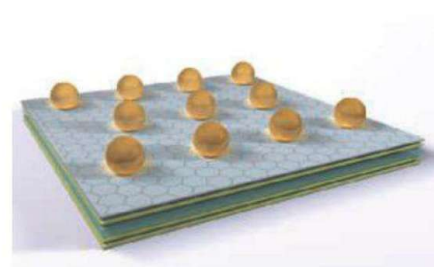
- 1303 Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge**
S. Mirceta et al.
 Increasing the number of charged amino acids allows for higher myoglobin concentrations in the muscles of diving mammals.
Research Article Summary; for full text:
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N. K. Grady et al.
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- 1311 Strong Light-Matter Interactions in Heterostructures of Atomically Thin Films**
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- 1314 Redox Heterogeneity in Mid-Ocean Ridge Basalts as a Function of Mantle Source**
E. Cottrell and K. A. Kelley
 Subducted carbon from ancient oceanic crust results in a more reduced mantle.
- 1317 Hydrogen Isotopes in Lunar Volcanic Glasses and Melt Inclusions Reveal a Carbonaceous Chondrite Heritage**
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- 1320 Clarifying the Dominant Sources and Mechanisms of Cirrus Cloud Formation**
D. J. Cziczo et al.
 Mineral dust and metallic particles initiate most ice nucleus condensation during cirrus cloud formation.
- 1324 Epistasis Among Adaptive Mutations in Deer Mouse Hemoglobin**
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 Deer mice have discovered that mutations distant from the oxygen-binding site help them live at high altitude.
- 1327 Root Effect Hemoglobin May Have Evolved to Enhance General Tissue Oxygen Delivery**
J. L. Rummer et al.
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Y. V. Zhang et al.
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- 1338 Parallel Neural Pathways Mediate CO₂ Avoidance Responses in *Drosophila***
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- 1342 Multisensory Control of Hippocampal Spatiotemporal Selectivity**
P. Ravassard et al.
 Virtual reality reveals how sensory cues differentially influence brain activity involved in sensing place in rats.
- 1346 Subangstrom Resolution X-Ray Structure Details Aquaporin-Water Interactions**
U. Kosinska Eriksson et al.
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 >> *Perspective p. 1294*



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Holding Your Breath >>

Hemoglobin and myoglobin are widely responsible for oxygen transport and storage (see the Perspective by **Rezende**). The ability of diving mammals to obtain enough oxygen to support extended dives and foraging is largely dependent on muscle myoglobin (Mb) content. **Mirceta et al.** (p. 1303) found that in mammalian lineages with an aquatic or semiaquatic lifestyle, Mb net charge increases, which may represent an adaptation to inhibit self-association of Mb at high intracellular concentrations. Epistasis results from nonadditive genetic interactions and can affect phenotypic evolution. **Natarajan et al.** (p. 1324) found that epistatic interactions were able to explain the increased hemoglobin oxygen-binding affinity observed in deer mice populations at high altitude. In mammals, the offloading of oxygen from hemoglobin is facilitated by a reduction in the blood's pH, driven by metabolically produced CO₂. However, in fish, a reduction in blood pH reduces oxygen carrying capacity of hemoglobin. **Rummer et al.** (p. 1327) implanted fiber optic oxygen sensors within the muscles of rainbow trout and found that elevated CO₂ levels in the water led to acidosis and elevated oxygen tensions.

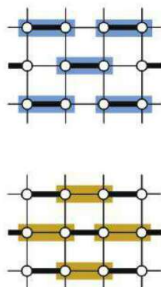


The Brain Speaks

The human brain, although overall bilaterally symmetric, shows asymmetry in certain functions such as speech. **Bishop** (p. 1302) reviews what is known about lateralization of the brain and acquisition of language, focusing on specific language and literacy impairments. Noninvasive imaging techniques have advanced our knowledge of how the brain develops during childhood and suggest that brain lateralization may not be as stable or as determinative as previously thought.

Fermionic Quantum Magnetism

Optical lattices loaded with cold atoms have been used successfully as quantum simulators of condensed matter systems; however, in the case of fermionic quantum magnetism, achieving low enough temperatures has been a major obstacle. **Greif et al.** (p. 1307, published online 23 May; see the Perspective by **Porto**) selectively tuned the exchange interactions in an optical lattice of fermions, forcing a redistribution of entropy such that in the low-entropy subsystem the effective temperature was sufficiently low enough to lead to magnetic correlations.



lavas at mid-ocean ridges. Because chemical and physical processes in the mantle change over time as a response to the availability of oxygen, the redox state of mid-ocean ridge basalts may trace the history of recycling between crust and mantle. **Cottrell and Kelley** (p. 1314, published online 2 May) analyzed the relation between the oxidation state of iron in a global suite of mid-ocean ridge basalts and tracers for mantle source composition. Over tectonic time scales, the recycling of reduced carbon in ancient crustal sediments may result in the preservation of more reduced zones in the mantle.

Dusty Origins

The formation of cirrus clouds begins with the production of ice nuclei, on which water vapor then condenses. **Cziczo et al.** (p. 1320, published online 9 May) determined the kinds of particles on which cirrus ice crystals form by sublimating samples collected by research aircraft and analyzing the chemical and physical properties of the residual seeds. Most of the seed particles were either mineral dust or metallic.

Common Water

The Moon has been traditionally considered bone-dry, but in recent years a number of studies have shown that during mantle melting, the lunar mantle had as much water as Earth's upper mantle. **Saal et al.** (p. 1317, published online 9 May; see the cover) measured the isotopic composition of hydrogen dissolved in volcanic glass and olivine-hosted melt inclusions recovered from the Moon by the Apollo 15 and 17 missions. Lunar magmatic water was indistinguishable from the bulk water in carbonaceous

chondrites and similar to terrestrial water, implying a common origin for the water contained in the interiors of Earth and the Moon.

Methylation and Methuselah?

Hutchinson-Gilford progeria syndrome (HGPS) and other prelamin A-associated progeroid disorders arise when farnesylated and methylated forms of prelamin A accumulate at the nuclear envelope. **Ibrahim et al.** (p. 1330, published online 16 May; see the Perspective by **Johnson**) show that reducing the activity of the isoprenylcysteine carboxyl methyltransferase (ICMT) mislocalizes prelamin A, triggers prelamin A-dependent AKT-mTOR signaling, and eliminates disease phenotypes in 30-week-old progeria model mice. Reduced ICMT expression increased the proliferation and delayed the premature senescence of progeria model mouse fibroblasts and cells from children with HGPS.

A Sense of Place

Hippocampal place cells are believed to be mainly governed by visual and self-motion cues. However, the contribution of sensory cues such as smells, sounds, and textures, etc., is difficult to eliminate. In virtual reality, these cues will not provide any information about the animals' position. **Ravassard et al.** (p. 1342, published online 2 May) developed a virtual-reality system as immersive and close to the real world as possible and compared place cells in rats running in this apparatus and in the real world. Twice as many neurons were active in a real-world situation compared to virtual reality. While place cells in the real world encoded position, place cells in a virtual world encoded distance.

CREDITS (TOP TO BOTTOM): THINKSTOCK, GREIF ET AL.



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A Perverted View of “Impact”

SCIENTISTS OFTEN FACE VEXING PROFESSIONAL DECISIONS: WHOM TO HIRE, WHAT TO FUND, WHAT TO PUBLISH, and whom to promote. Because science is about the unknown and its greatest discoveries are often the least expected, scientists often have little to go by except intuition and experience. For this reason, a seductively simple template has recently been introduced: assessment based on “impact and significance.” Thus, the U.S. National Institutes of Health has elevated “significance” to an explicit criterion in funding decisions. It requires that grant reviewers write a paragraph on “impact,” which it defines as the likelihood that the proposed work will have a “sustained and powerful influence.”* Especially in fundamental research, which historically underlies the greatest innovation, the people doing the work often cannot themselves anticipate the ways in which it may bring human benefit. Thus, under the guise of an objective assessment of impact, such requirements invite exaggerated claims of the importance of the predictable outcomes—which are unlikely to be the most important ones. This is both misleading and dangerous.

One may be able to recognize good science as it happens, but significant science can only be viewed in the rearview mirror. To pretend otherwise distorts science. DNA restriction enzymes, once the province of obscure microbiological investigation, ultimately enabled the entire recombinant DNA revolution. Measurement of the ratios of heavy and light isotopes of oxygen, once a limited area of geochemistry, eventually allowed the interpretation of prior climate change. What is now promoted as high-impact science is usually a narrow extension of existing experimental designs in a program focused on a set of feasible goals. Fuzzy new directions that might fail, but could open up major new questions, are often dismissed as too speculative and considered low-impact. And in biomedical science, there is an increasing tendency to equate significance to any form of medical relevance. This causes biochemical investigations and research on nonmammalian systems to be treated as intrinsically less valuable than studies on human cells. As a result, biomedicine is losing the historically productive cross-fertilization between model systems and human biology.

In science, faster, better, and cheaper are not as important as conceptual, novel, and careful. Focusing resources narrowly on areas that are deemed impactful, while ignoring many others, decreases diversity, making science less productive. Assessments based primarily on impact may also be contributing to an apparent epidemic of irreproducible results in the biomedical literature. Reviewers and editors increasingly insist on major extensions of the submitted work in order to inflate its (narrowly defined) impact, while at the same time making such extensions a condition for acceptance. In today’s competitive job and grant market, these demands create a strong inducement for sloppy science.

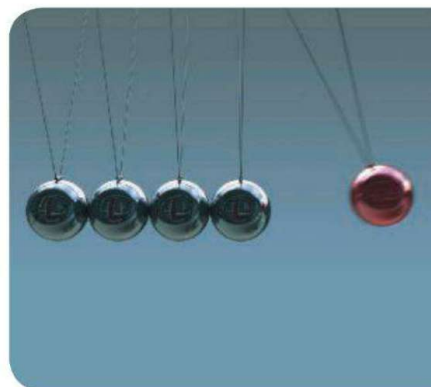
What ails science today requires an honest diagnosis. Scientists are failing to live up to the trust society has placed in them. The scientific community must create leadership with the courage and independence to take control of the structure of its training, the peer-reviewing of its journals, the organization of grant review panels, and the overall priorities that are set. There are strong political, economic, and institutional interests that are not shy about asserting themselves. Scientists have to be equally assertive and even more persuasive.

I also believe, along with Huda Zoghbi,[†] that scientists must challenge the assumption that translation, rather than fundamental understanding, is the choke point of progress in the application of science to societal problems. They should work hard to encourage risk and exploration, while at the same time rewarding careful, thoughtful investigation. And they should reemphasize humility, banishing the words “impact” and “significance” and seeing them for what they really are: ways of asserting bias without being forced to defend it.

— Marc Kirschner

10.1126/science.1240456

*http://grants.nih.gov/grants/peer/guidelines_general/impact_significance.pdf. [†] H. Y. Zoghbi, *Science* **339**, 250 (2013).





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ASTRONOMY

Cosmic Correlation

The cosmic infrared background, the integrated infrared light produced by all extragalactic sources in the universe, has been found to exceed the expected emissions from known galaxies, including the most distant ones. To understand the nature of the populations responsible for this excess, Cappelluti *et al.* cross-correlated the fluctuations in the infrared and x-ray backgrounds. The infrared background is sensitive to stellar populations, whereas the x-ray background probes radiation from accreting black holes and thermal x-ray emission from hot ionized gas. The

detected correlations indicate that at least 15 to 20% of the cosmic infrared background is produced by sources that are powerful x-ray emitters. Based on theoretical calculations, Yue *et al.* propose in a different study that the first cosmic black holes, which formed from direct collapse of the gas in the first galaxy halos, are responsible for the infrared background fluctuations. This hypothesis is consistent with the observed correlation between the infrared and x-ray backgrounds. — MJC *Astrophys. J.* **769**, 68; (2013) *Mon. Not. R. Astron. Soc.* **10.1093/mnras/stt826** (2013).

PHYSICS

Unexpected Conductivity

Interfacing two dissimilar crystals can lead to unexpected phenomena, as best illustrated by the formation of a highly mobile metallic two-dimensional electron gas (2DEG) between two insulators, SrTiO_3 (STO) and LaAlO_3 . The interface is usually formed using the (100) crystallographic orientation of the substrate, STO, and the conductivity of the 2DEG is explained through the polarization catastrophe model, in which the discontinuity of the polarization at the interface leads to charge transfer. Annadi *et al.* studied what happens when the (110)-oriented STO is used as a substrate instead. Naïvely, one may expect that in this case the interface would always be insulating, because there is no polarization discontinuity; however, the researchers found that the transport properties of such interfaces are similar to those formed on (100) surfaces. One exception to that similarity was the appearance of anisotropic conductivity, attributed to the differing properties of the Ti-O-Ti chains along the two in-plane directions. First-principles calculations suggested that the unexpected conductivity could be explained through the model of an energetically stable buckled interface, which, contrary to expectations, did induce a polarization discontinuity. — JS

Nat. Comm. **4**, 1838 (2013).

VIROLOGY

A Careful Escape

Viruses gain entry to host cells through binding to specific receptors, which subsequently mutate to block virus binding. This can be problematic, however, because many viral receptors perform essential functions for their hosts. One such example is transferrin receptor 1, which regulates iron uptake by host cells. How are such mutations

selected so as to not disrupt the essential function of the receptor?

Demogines *et al.* now show how a small amount of mutations in transferrin receptor 1 can alter viral host specificity.

Analysis of the ratio of synonymous to nonsynonymous DNA mutations in the rodent transferrin receptor identified a handful of residues that were under positive selection. These residues corresponded to the region of the receptor that interacts with the virus rather than those residues necessary for iron uptake. A search for human single-nucleotide polymorphisms identified transferrin receptor variants that reduced viral uptake in cultured human cells but maintained iron regulation. These results demonstrate that in the constant arms race between viruses

and the cells they infect, positive selection of residues involved in viral entry can be divorced from regions of the receptor that are essential for host function. — PJH

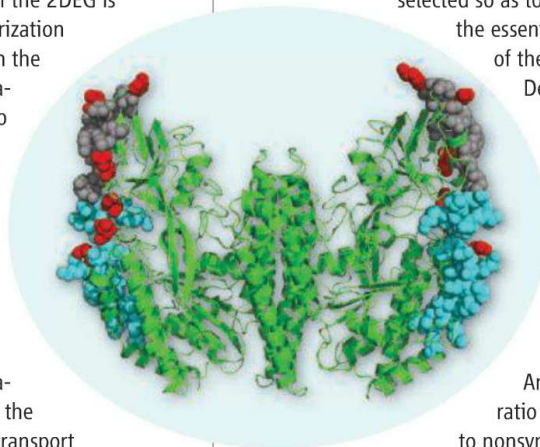
PLoS Biol. **11**, e1001571 (2013).

BIOCHEMISTRY

Handily Handling Nitrite

Nitrogen is, of course, an essential element for life; fortunately, humans have no need to worry about its various oxidation states because we acquire it in readily usable forms (such as amino acids) in our diet. Microbes do the hard work of reducing dinitrogen and handling ammonium, nitrite, and nitrate ions. Crystal structures of some of the key enzymes and transporters have provided insights into the biochemistry of these small molecules, and Zheng *et al.* offer the latest step forward by presenting the 2.6 Å structure of *Escherichia coli* nitrate/nitrite transport protein NarK. They find the familiar 12-transmembrane helices of a major facilitator superfamily transporter, and by soaking in nitrite, they established the location of the active site, which almost certainly is also where nitrate binds. The anionic substrates are locked into place by two opposing arginine residues, and phenylalanines constitute the floor and ceiling of this cage. How reciprocal conformational changes are triggered by the binding of cytoplasmic NO_2^- versus the binding of extracellular NO_3^- is as yet unclear, but these two anions differ in their shape (vee versus trigonal) and their distribution of partial charge, so there are lots of ways for NarK to do what proteins do best. — GJC

Nature **497**, 647 (2013).



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AROUND THE WORLD



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Safety Agency Backpedals
On Premie Study

Under fire from researchers, the U.S. government agency responsible for protecting human research subjects has shelved a 7 March decision to sanction the leaders of a clinical trial involving premature infants for

not fully disclosing its risks (*Science*, 19 April, p. 254). “We have put on hold all compliance actions,” the U.S. Office for Human Research Protections (OHRP) announced in a 4 June letter to the University of Alabama, Birmingham, which led the trial. OHRP’s move came a day before *The New England Journal of Medicine* published two pieces



Breath of life. Study examined oxygen given to premature infants.

arguing the agency overreacted. OHRP says that it plans to hold a public meeting on the matter, with an eye toward clarifying risk disclosure rules. <http://scim.ag/premiestudy>

Beijing and Washington, D.C. 2

Reducing HFCs Under
Montreal Protocol

Breaking a policy logjam, China and the United States agreed on 8 June to work for deep global cuts in the use of potent atmospheric warming chemicals called hydrofluorocarbons (HFCs). The 1989 Montreal Protocol to protect the ozone layer boosted the use of HFCs as coolants in appliances,

because they do less damage than chlorofluorocarbons. As HFCs’ role in warming became clear, nations squabbled over whether to use the protocol or a new climate agreement to reduce use; now, the two powers have agreed to pursue reductions under the protocol. The White House estimates that cuts could erase greenhouse emissions equivalent to 90 gigatons of CO₂ by 2050, or nearly 2 years’ worth of current global emissions.

Stockholm 3

New Home for Nobel Prize

Sweden’s biggest research foundations have secured 800 million kronor (\$122 million) to build a new Nobel Center in Stockholm that will bring the Nobel Museum, the Nobel Foundation, and Nobel Prize ceremony under one roof. The museum and foundation now have separate addresses, and the prize is awarded at the Stockholm Concert Hall. Come 2018, however, all would be united in the new venue. An architect for the project will be chosen in a global competition that enters its final phase this week.

The Nobel Foundation welcomed the 4 June announcement that the Knut and Alice Wallenberg Foundation and the Erling-Persson Family Foundation (run by the chairman of fashion retailer H&M) had raised the necessary funds. The center will “generate scientific activities of the highest international caliber,” says Lars Heikensten, the Nobel Foundation’s executive director.

Rome 4

Rally to Defend Science

A flash mob of 30 researchers calling themselves Italy United for Correct Scientific Information appeared on the Spanish Steps of Rome on 8 June to protest an attack



Rallying cry. Researchers protest misinformation about science at Rome’s Spanish Steps.

against an animal facility at the University of Milan in April. It was part of a series of protests and conferences in 15 cities across Italy this month to protest what organizers say is an antiscientific attitude in Italy and widespread “misinformation” about science in the media.

“We want to show that we do not live in an ivory tower,” says Dario Padovan, a biologist at the University of Trieste. “We are not afraid to defend our research and understand the need of communicating it correctly.” Press coverage of April’s attack, or of the conviction of Italian researchers for their failure to warn about the risk of a deadly earthquake in L’Aquila, shows that Italian media tend to focus on the emotional side of a story and fail to delve into the scientific facts, says Federico Baglioni, one of the organizers of the 8 June event. <http://scim.ag/italyrally>

NEWSMAKERS

Three Q’s

Last week, more than 70 research, health care, and patient advocacy organizations announced a “global alliance” to help researchers securely share genome sequences and clinical information. **David Altshuler** of the Broad Institute in Cambridge, Massachusetts, is leading the planning. <http://scim.ag/Altshuler>



Q: Why is this alliance needed?

D.A.: We have to be in a position to compare genomes and clinical data if we want to learn and ... help people, like give them accurate predictions or learn the biology of a disease. It’s going to take millions of genomes. Even in a given disease there are often many different genes that can play a role, and there are many, many different diseases.

BY THE NUMBERS

\$65 Amount added to the U.S. economy per \$1 of the \$12.3 billion federal investment in genomics-related research, according to Battelle Technology Partnership Practice and United for Medical Research Group.

\$35 per metric ton The “social” cost of carbon emissions—an estimate of climate change damages to health, property, and agriculture—up from \$21 per metric ton, per a statement last week by the U.S. Office of Management and Budget. All federal agencies must use the new number in regulations.

\$244 billion Amount of global investments in renewable energy in 2012, increasingly by developing countries.

Q: Will you essentially merge different databases?

D.A.: That’s not what this is about. We’re inspired by the example of the World Wide Web and also the Human Genome Project. The idea is to focus on standards and shared principles and ethics that would make it possible for many people to build things that would be individually innovative and yet collectively could learn from each other.

Q: Have any of the big biobank projects, such as Kaiser Permanente and Vanderbilt, declined to participate?

D.A.: They’ve not turned us down. There are only so many organizations that have thus far been part of the discussion ... whether or not those organizations choose to participate will be up to them.

New Max Planck President

Martin Stratmann, 59, who studies interface chemistry, surface engineering, and corrosion, was elected the new president of the Max Planck Society, one of Germany’s main research organizations, on 6 June. Elected to a 6-year term today at the society’s annual meeting in Potsdam, Stratmann will replace current president, developmental biologist Peter Gruss, next June.

Random Sample

Lack of Sleep Leads To Award-Winning Screenplay

In the dystopian world of *Waking Hours*, a screenplay by University of California, Los Angeles (UCLA), film student Barnett Brettler, a disease called fatal insomnia doesn’t just rob victims of sleep until they die of exhaustion. It “takes away their façade” by revealing their true emotions, he says.

In real life, fatal insomnia is rare, produced by misfolded brain proteins. But in Brettler’s script the world is overrun by its zombielike victims. Elwin, a border patrol agent in the United Kingdom, is charged with keeping the diseased out—but faces a dilemma when his scientist girlfriend wants to leave to help treat victims on the mainland.

The idea occurred to Brettler during a program sponsored by the Alfred P. Sloan Foundation, which pairs young filmmakers with scientists. He worked with UCLA microbiologist Imke Schröder, whose enthusiasm for the project, he says, was “contagious.”

Now, Brettler has a chance to turn his screenplay into a feature film. In April, he received the Sloan Student Grand Jury Prize, an annual \$50,000 award to help produce a student screenplay with a science theme. According to Sloan Vice President of Programs Doron Weber, the foundation has spent \$3.5 million over the past 15 years to encourage young filmmakers to “be more open to the idea that there’s good raw material in science and technology.”



Rising star. Student filmmaker Barnett Brettler (third from left) received \$50,000 for his screenplay, *Waking Hours*.

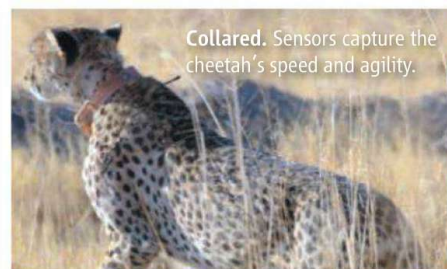
Stratmann is one of the directors at the Max Planck Institute for Iron Research in Düsseldorf and has been a vice president of the society since 2008. The society, which had a budget last year of €1.5 billion, comprises 80 institutes and research facilities and employs more than 5000 scientists. <http://scim.ag/Stratmann>



FINDINGS

Agility, Not Speed, Puts Cheetahs Ahead

The fastest land animal on Earth depends on more than speed to catch its prey; instead, much of the cheetah’s hunting success comes from its ability to slow down and turn quickly. Using a radio collar equipped with GPS, accelerometers, and other devices that could relay a cheetah’s position, velocity, and direction 300 times each second, biomechanist Alan Wilson from the Royal Veterinary College in London and his colleagues tracked the movements of five wild cheetahs in Botswana, recording 367 hunting

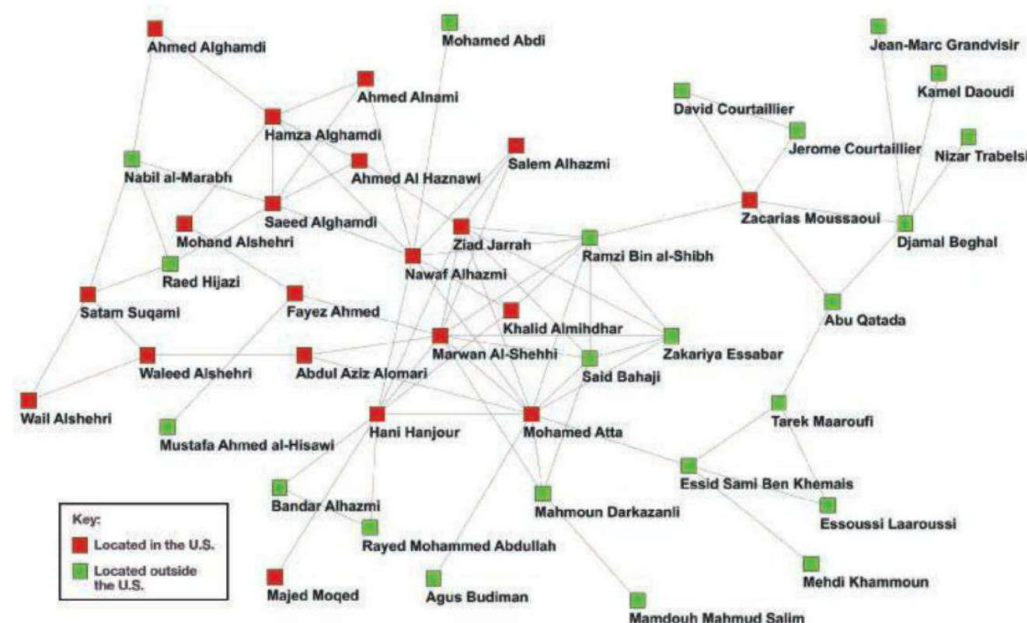


Collared. Sensors capture the cheetah’s speed and agility.

runs in 18 months. Cheetahs have four times the muscle power—and acceleration—of humans, can slow down by 4 meters per second in a single stride, and can successfully hunt in densely wooded areas as well as in open grasslands, the team reported this week in *Nature*. The collars are “a big step forward in terms of understanding what animals do in the real world,” says David Carrier, a comparative biomechanist at the University of Utah in Salt Lake City.

Science LIVE

Join us on Thursday, 20 June, at 3 p.m. EDT for a live chat with experts on **the use and misuse of science in summer blockbusters**. <http://scim.ag/science-live>



COMPUTER SCIENCE

Network Science at Center Of Surveillance Dispute

Last week, civil libertarians cried foul when press reports revealed that, in its efforts to ferret out terrorists, the U.S. National Security Agency (NSA) is collecting cell phone records and Internet data from companies such as Verizon, Facebook, and Skype. Some argued that the federal government is spying on its own citizens. From the nature of the data, scientists say it's clear that NSA is performing network analysis, a type of science that aims to identify social groups from the connections among people. And NSA is hardly the only organization doing such work, researchers say. Private companies are already tracing people's social circles.

"I can tell you that this kind of thing is extremely effective," says Alex Pentland, a computational social scientist at the Massachusetts Institute of Technology in Cambridge who has studied phone networks.

Born of the social sciences decades ago, network science blossomed in the 1990s thanks to the confluence of mathematical tools developed by theoretical physicists and huge data sets produced by cell phones, the Internet, and other digital technologies. Network methods have entered the mainstream in fields such as epidemiology, in which researchers use data such as airline networks to help model the spread of disease.

NSA's network analyses likely involve four levels of inquiry, says Brian Uzzi, a soci-

ologist at Northwestern University in Evanston, Illinois. Suppose an analyst works with only phone data. The first step, Uzzi says, would be to map who calls whom—with each person represented by a point or "node" and each person-to-person link represented by a line or "edge"—to create a simple communication network. Next, the analyst would study details of calls—their frequency, duration, and timing—to determine how closely connected each pair of people is. This step breaks the communication network into smaller, overlapping social networks.

The third step would be to study the dynamics of a social network, to see how activity ebbs and wanes and the network evolves. The fourth step would be to try to correlate the dynamics of the network with external events, Uzzi says, such as terrorist bombings in Iraq or Afghanistan. In reality, of course, analysts work with data from many different sources to try to trace a social network as accurately as possible.

Given enough data from electronic and other sources, one can identify a social group, be it an al-Qaeda cell or a classic car club. But the methods have limitations. It's unlikely that NSA is trying to decipher the social networks of all 300 million people in the United States, scientists say, as such an effort would yield an impenetrable hair ball.

Instead, NSA is most likely fleshing out

Connect the dots. This network links the attackers of 11 September 2001 and alleged associates.

the social circles of suspects identified by other means. "If you have the data on everyone, then when you have reason to believe that Mr. X is someone of interest, you can go back and look at his behaviors," says David Lazer, a political scientist at Northeastern University in Boston.

NSA is hardly the only organization tracing social networks. Such work is done by the companies that generate the data, researchers say, and some, such as Twitter, sell their data. Valdis Krebs, a network scientist and the founder of Orgnet LLC in Cleveland, Ohio, says he did work for a phone company that worried its most highly connected subscribers might leave and take their contacts with them. The company hired Krebs to help identify those key customers so that they might receive perks—a task not dissimilar to unmasking the leaders of terrorist networks. "It's the same modeling, but where one group is looking for the nodes to get rid of, the other is looking for the ones to butter up," Krebs says.

Some scientists say that concerns over the NSA program are overblown. "Why do people get upset if the government is handling the data but not if a company is handling the data?" says Alessandro Vespignani, a physicist at Northeastern who, with support from the National Institutes of Health, has helped predict the spread of influenza. Uzzi says the government has its hands full hunting terrorists. "With all that going on, will they come after you and me?" he says. "It just doesn't make any sense."

But some see darker potentialities. Krebs that says his grandparents and parents lived in Latvia and suffered through the brutal repression of the Nazi and Soviet regimes. A modern-day Hitler or Stalin could use network analysis to target political opponents, he says. "People like that get this technology and it's over," he says. "This is the best way to find you and eliminate you." Lazer shares that concern. "It's not the government now, it's the government in 20 years or 40 years that one has to worry about," he says.

Others say that politically, the U.S. government is practically obligated to employ such techniques. If NSA didn't use them and another major terrorist attack occurred, then people would complain that the government hadn't done all it could to prevent the attack, Vespignani says: "We have the tools and we have the information. We have to use them. It's as simple as that."

—ADRIAN CHO

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SOCIAL SCIENCE

Bold Plan, Uncertain Future For Gun Violence Research

After 2 decades of giving a cold shoulder to scientists who study the public health impact of gun violence, the U.S. government is being urged to give them a warm embrace. A report last week from the National Research Council (NRC) and the Institute of Medicine (IOM) lays out a national strategy for firearms research. Now the debate begins on how to implement it.

Estimates of U.S. firearms-related casualties top 30,000 deaths per year and twice as many nonfatal injuries, the highest rate among industrialized nations. That should place it squarely in the domain of the Centers for Disease Control and Prevention (CDC), says Jeffrey Runge, a professor of medicine at the University of North Carolina, Chapel Hill, and one of the report's authors. "Firearms violence has all the features of a big public health problem," he says.

But CDC has long been relegated to the sidelines. In the 1990s, under pressure from the National Rifle Association (NRA), Congress effectively gutted CDC research on the public health impact of guns by cutting precisely the same amount of funding allocated to the program—\$2.6 million—and passing legislation that prohibited the agency from conducting research that might "advocate or promote gun control." In the wake of several mass murders with firearms last year, including one attack that killed 20 schoolchildren, President Barack Obama called for lifting that ban and asked Congress to give CDC \$10 million for research (*Science*, 25 January, p. 381). The White House also asked for advice from NRC and IOM, which organized a panel of firearms experts, criminologists, and public health scientists.

Their 5 June report names more than a dozen "high priority topics that could be explored with significant progress" expected in 3 to 5 years. But the report's overarching theme is the need for data. "The problem is there just aren't any data," says report committee chair Alan Leshner, a mental health expert and CEO of AAAS (publisher of *Science*).

There are two major gaps. First, details about the circumstances of firearms-related casualties are often lacking. Second, the number of guns in private hands across the country—

legally or illegally—is simply unknown.

Closing the first gap will require improvement and coordination of existing data sets, Runge says. He points to research on traffic-related casualties conducted during his tenure as director of the U.S. National Highway Traffic Safety Administration as a model of what can be done. Based on insights gleaned from statistical analysis of state and national data-

How many? It's hard to get data on guns in the United States, making it difficult to study their use.



bases—such as the need to lower the center of mass in SUVs—safety measures reduced the number of traffic deaths per year from 43,000 in 2001 to 31,000 in 2005, he says.

Although research expressly focused on guns has been stymied, CDC has collected data through its National Vital Statistics System, which includes a tally of gun-related deaths. "These data are based on death certificates and represent a good count of gun deaths across the U.S. from all causes—suicide, homicide, and unintentional gun deaths," says Charles Branas, an epidemiologist at the University of Pennsylvania, who

has used them to compare urban versus rural gun deaths. A newer database, the National Violent Death Reporting System, includes far more detail about each gun-related fatality, Branas says, but so far "it only covers 18 states."

Closing the second data gap—mapping gun distribution—is far trickier. NRA fiercely opposes a gun registry. There's so much mistrust of monitoring gun commerce that even inserting electronic safety measures in handguns—something the report names as a research priority—is seen by some people as "a thinly veiled conspiracy to allow the government to track weapons for the ultimate purpose of confiscation,"

says Donald Sebastian, a chemical engineer at the New Jersey Institute of Technology in Newark. Runge is adamant that public health research on gun violence doesn't need an owner registry. "We don't care" what you own, he says. "We only care when a fatality occurs. That's when the data collection gets triggered." Survey methods not involving gun registries, Branas adds, "could perhaps get us serviceable estimates" of gun distribution.

How CDC will pay for the research, and whether Congress and the House of Representatives will support it, are two pressing unanswered questions, says Arthur Kellermann, a firearms policy researcher at RAND, a Santa Monica, California-based think tank. Aside from the \$10 million requested by the White House, no new funding for gun violence research is on the table. And if Congress doesn't strike down the restrictive 1996 legislation banning research that promotes gun control, the research agenda will remain in limbo, he says. What are the odds Congress will strike that clause? "My guess is slim to none," Kellermann says.

CDC declined an interview request, saying that the agency "looks forward to reviewing the report." Kellerman understands why officials are being careful. "Many of us consider [CDC] the most highly regarded public health agency in the world," he says. He hopes it doesn't get caught like "a rag doll between a determined White House and an equally determined Congress."

—JOHN BOHANNON

With reporting by Emily Underwood.

U.S. STEM EDUCATION

Educators, Lawmakers Question Proposed Reorganization

The status quo is a powerful force in Washington, D.C. And that resistance to change could be good news for Tufts University neuroscientist Karina Meiri and other science educators battling to derail a White House proposal to revamp the federal government's \$3 billion investment in STEM (science, technology, engineering, and mathematics) education.

Last week, Republican and Democratic legislators used a House of Representatives science committee hearing to lambaste the administration's plan, calling it "rushed" and "not well thought out." That bipartisan pushback could turn what has been a Sisyphean challenge for Meiri and other critics into a possibly winnable fight.

At issue is a proposal to shrink by more than one-half the 226 STEM programs now funded by a dozen agencies (*Science*, 19 April, p. 258). It would concentrate resources at three agencies: the Department of Education for elementary and secondary school programs, the National Science Foundation (NSF) for undergraduate and graduate programs, and the Smithsonian Institution for informal and public science activities. Administration officials say that eliminating 78 programs and merging 48 others would save an estimated \$176 million and enable them to redistribute the money to the three lead agencies. They also want to create 10 programs and boost overall STEM spending by \$195 million.

The reorganization, part of the president's 2014 budget request to Congress, is essential "to free up resources for the administration's highest [STEM education] priorities," John Holdren, the president's science adviser, testified at the 4 June hearing. And while lawmakers on the science committee, the first to examine the plan in depth, generally agreed that federal STEM education programs are not well coordinated and that 226 programs is probably too many, they didn't like how the White House wants to reshuffle the deck.

Several lawmakers reported that their constituents are upset about proposed cuts of as much as 33% in STEM education budgets at so-called mission agencies. These agencies, including NASA, the National Institutes of Health (NIH), the Department of Energy, and the National Oceanic and Atmospheric Administration, exist for purposes other than

to prepare STEM workers. But over the years, they have tapped their scientific expertise to develop education programs for students, teachers, and the general public.

"I hope our witnesses can tell us what was wrong with the programs the administration wants to cut or consolidate," said committee chairman Representative Lamar Smith (R-TX) at the start of the hearing. Committee members then spent 2.5 hours peppering Holdren, NSF's Joan Ferrini-Mundy, and NASA's Leland Melvin with questions about how the White House decided to eliminate programs, whether it had consulted outside

cer, infectious diseases, and diabetes. But the White House has marked for extinction both the Science Education Partnership Award (SEPA) program that funds her and dozens of other projects as well as NIH's Office of Science Education, which manages SEPA.

Meiri admits that she's a novice when it comes to working the corridors of power in Washington. But after hearing about the realignment, she decided to register her concerns by going straight to the top—or at least as close as she could get. In two e-mails and one phone conversation last month with the White House Office of Public Engagement, Meiri aimed for what she hoped was the plan's Achilles' heel.

"The decision to drop health science from the national education agenda could be politically embarrassing to the president," she argued. Efforts to curb spending under the nation's new health care law, known as Obamacare, require an informed citizenry, she explained. And SEPA is the only federal program that funds academic scientists sharing the latest health science results with the public.

Holdren's answer to a question from Representative Suzanne Bonamici (D-OR) about the impact of ending SEPA suggests that Meiri's concern about the fate of health science education is well-founded. "We are determined to figure it out in a manner that would not lose the effectiveness of the engagement programs that already exist," Holdren said.

Eliminating NIH's STEM programs will reduce incentives for biomedical scientists to work on education, warns J. Michael Wyss, chair of the American Physiological Society's education committee and a neurobiologist at the University of Alabama, Birmingham. "Right now, the country is gaining access to tremendous scientific resources for free," says Wyss, a former SEPA grantee. "And I'm not sure the average PI [principal investigator] would be interested if the money came from the Department of Education rather than from NIH." He also questions the wisdom of a strategy based on "moving the mission but without the money."

Theresa Schwerin, who manages education programs for the Institute for Global Environmental Strategies, a NASA contractor, sees the same thing happening in the space sciences if NASA cancels the education and



Endangered species. An exhibit on the connection between health and evolution, now at the New York Hall of Science, was funded by an NIH program targeted for elimination.

STEM experts, and whether the lead agencies were up to the job.

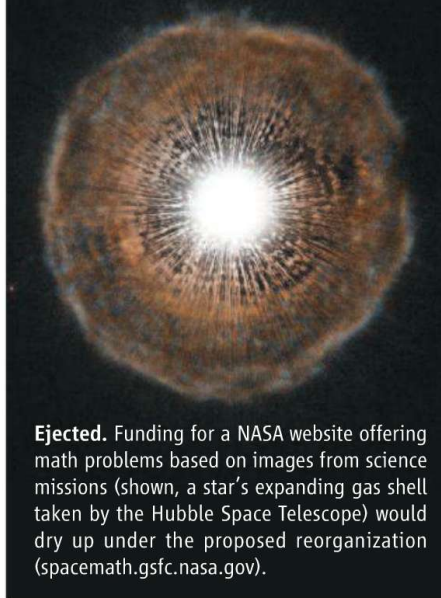
On 31 May, an interagency committee reporting to Holdren's Office of Science and Technology Policy unveiled a 5-year strategic plan for federal STEM education. The science panel's top Democrat, Representative Eddie Bernice Johnson of Texas, suggested that the reorganization take a back seat to the strategic plan, which doesn't mention any cuts. "My hope is that the strategic plan can serve as a new starting point for more sensible and well thought out implementation steps in 2015 and beyond," Johnson said.

Such comments are sure to bolster efforts by Meiri and other science educators to rally support for existing programs. Meiri runs an NIH-funded project that exposes high school students in the Boston area to the latest research on health scourges such as can-

outreach components of its scientific activities. “How will scientists from the science mission directorate be engaged in education and public outreach if there are no, or very little, resources available for this?” she asks. “Are they expected to be volunteers? This is just not a realistic or effective approach.”

Putting all of NASA’s education eggs in one basket—the education office that Melvin runs—will still leave sufficient resources “to make sure we can bring forward the best [STEM education] programs,” Melvin told Representative Donna Edwards (D-MD). But he confirmed her supposition that he did not propose the reshuffling at NASA and had not provided his federal colleagues with a list of what should be axed.

The reorganization’s critics hope that Congress will decide to stop the White House plan in its tracks. They’ll need more than the House science committee on their side, however, because spending falls under



Ejected. Funding for a NASA website offering math problems based on images from science missions (shown, a star’s expanding gas shell taken by the Hubble Space Telescope) would dry up under the proposed reorganization (spacemath.gsfc.nasa.gov).

the purview of the powerful appropriations panels.

NIH-funded science educators face an even tougher challenge, namely, avoiding the dismantling of the agency’s science edu-

cation infrastructure. Lawrence Tabak, principal deputy NIH director, acknowledges that NIH is the only federal agency that supports health science education. And he says that his colleagues at other agencies are trying to figure out “how NIH can provide the technical expertise that is needed to support programs of this type under the reorganization.” He agrees that such uncertainty “is hard on the community.” But every NIH program is undergoing similar intense scrutiny, he notes, after this spring’s government-wide budget cuts known as sequestration amplified the impact of years of flat budgets.

Tabak says that “no decision has been made” about the fate of the Office of Science Education, and the office’s longtime director, Bruce Fuchs, declined to comment. But most scientist-educators believe Fuchs’s position will disappear on 30 September, the last day of the 2013 fiscal year.

—JEFFREY MERVIS

U.S. SCIENCE POLICY

NSF Cedes Little Ground on Political Science Reviews

Facing a controversial congressional directive to fund only political science research that promotes national security or economic development, the U.S. National Science Foundation (NSF) has decided that it can follow those orders without deviating too much from its traditional peer-review process.

The new NSF policy, announced on 7 June, means that reviewers meeting this month to evaluate roughly 200 proposals in political science will still use the agency’s two long-standing criteria—intellectual merit and broader impacts. However, those proposals will receive a second review by another panel, which will apply the two criteria that Senator Tom Coburn (R-OK) added to a government-wide spending bill for 2013 (*Science*, 29 March, p. 1510). NSF program officers will consider both sets of reviews in deciding which proposals to fund.

“We take the congressional language very seriously,” says Myron Gutmann, head of the social, behavioral, and economic sciences directorate that includes the \$10-million-a-year political science portfolio. “We’ve thought long and hard about the new language, and we want to make sure that reviewers have the same chance.”

Adopted by voice vote without debate, Coburn’s amendment was meant to address the concerns of several legislators, who have asserted that NSF occasionally funds frivolous or trivial research that wastes taxpayer

dollars. Accordingly, the language requires the NSF director to issue a public explanation of each grant awarded.

Gutmann says that summaries posted on NSF’s website of every funded project already serve that purpose and that there’s no need for a statement from the director. Everything at NSF is done under the authority of the director, he adds, although much of the actual work is carried out by the agency’s 1250 employees. “Our goal is to operate within the law,” he explains. The extra layer of review could delay decisions normally made this summer until the fall, Gutmann notes.

Asked for a reaction, Coburn issued this statement through his press secretary: “Political scientists, of all people, should understand Congress’ responsibility to set priorities and make hard decisions. ... [W]e need to make sure we adequately invest in truly transformative research ahead of important but lower-priority research. I will continue to monitor NSF’s Political Science Program to ensure all funded programs meet the standards Congress passed in March.”

Many social scientists initially feared that NSF might scrap its entire political science portfolio this year after deciding that separating out proposals meeting Coburn’s narrow language would be impossible. NSF’s response has allayed some of those concerns. But it leaves open the question of how well peer reviewers can spot research that would

foster national security and economic development and whether NSF should adopt such criteria at all.

“This is a recipe for chaos via the imposition of ad-hoc, subjective, and incoherent opinion. How does that promote social science?” asks political scientist Jim Granato, a former NSF program manager now at the University of Houston in Texas.

Greg Koger, a political scientist at the University of Miami in Florida, shares Granato’s anger toward Congress, calling it “outrageous and disturbing that political science has been singled out for extra scrutiny and scorn.” But he hopes that NSF will use “a broad interpretation of national security and economic interests” in making funding choices.

NSF’s response to Coburn could also have implications for a proposal from the chairman of the House of Representatives science committee to apply similar language to NSF’s entire research portfolio (*Science*, 24 May, p. 911). The plan, floated by Representative Lamar Smith (R-TX), would require the NSF director to certify that all research that the agency funds is groundbreaking, not duplicative, and vital to U.S. interests. The language has roiled the scientific community, which sees it as an attack on the peer-review process. So NSF’s response to Coburn may also be a signal to Smith that it sees little reason to make any fundamental change to its peer-review process.

—JEFFREY MERVIS

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PHYSICS

Magnet on the Mighty Mississippi: A New Life for Muon Experiment

Your GPS navigator would beg you not to do it. If you ask it how to get from Upton, New York, to the western suburbs of Chicago, it will tell you to drive due west on Interstate 80 for 14 hours. It will *not* send you on a 6-week barge trip down the Eastern Seaboard, around Florida, across the Gulf of Mexico, and up the Mississippi.

Yet when a massive piece of equipment sets off this weekend from Brookhaven National Laboratory to its new home at the Fermi National Accelerator Laboratory (Fermilab), that meandering 5000-kilometer voyage is what lies in store. The device—the storage ring from a particle accelerator—is 15 meters across and weighs 14 tons. It's also more delicate than a porcelain tea set, which is why not just any route will do.

The ring formed part of an experiment called Muon $g-2$ (pronounced “g minus two”), which ran at Brookhaven from 1999 to 2001. By tracking how muons “wobbled” when trapped in the ring’s exceptionally uniform magnetic field, physicists could precisely measure a property of the particle called its magnetic moment (g). What’s more, by calculating a figure known as $g-2$, they could determine how any virtual particles that popped in and out of existence in the vacuum around a muon affected its magnetic moment. But when the Brookhaven experiment measured $g-2$ to a precision of 0.7 parts per million, its results didn’t agree with the standard model of particle physics—meaning that perhaps there were some undiscovered virtual particles interacting with its muons.

“We have one of the very intriguing, tantalizing hints of new physics,” says David Hertzog, a physicist the University of Washington, Seattle, and a co-spokesperson for Muon $g-2$. To count as definitive, however, the results needed to reach a standard deviation of 5 sigma. Muon $g-2$ maxed out at 3.6 sigma. Was the result a Nobel-worthy discovery, or merely a statistical fluctuation? “I felt like it was a scientific obligation to try to resolve this,” Hertzog says. “You don’t want to leave these kinds of things hanging.”

To repeat the experiment with higher precision, researchers needed more muons than Brookhaven’s accelerator could provide. Fermilab, meanwhile, was on the hunt for experiments that could make use of the high-intensity particle beams it planned to produce

after the Tevatron, its flagship accelerator, shut down in 2011. So scientists at both laboratories hatched a plan to move the experiment from the East Coast to the Midwest.

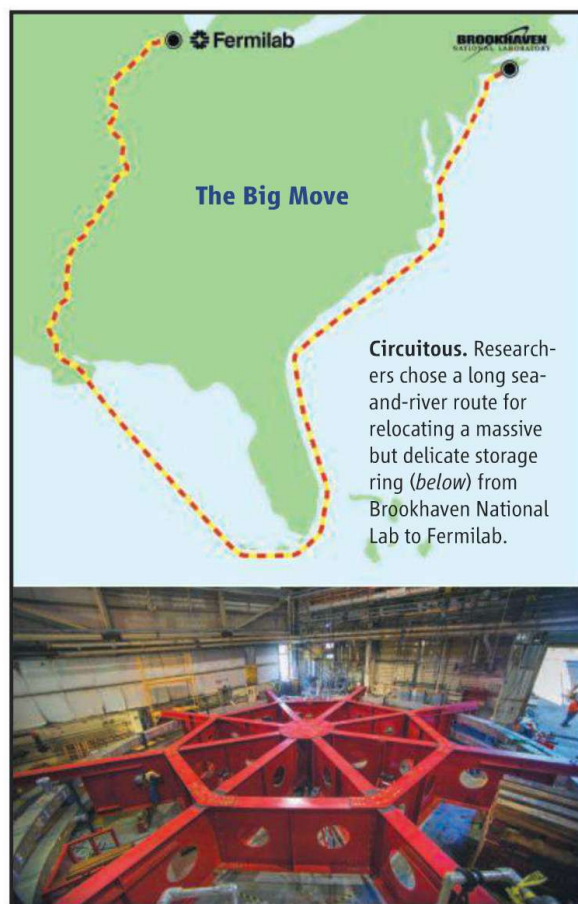
Some components, such as the steel supporting the storage ring, were easy to pack up and truck the 14 hours to Muon $g-2$ ’s new home. Others were outdated and would need to be built anew. But one piece of equipment at Brookhaven was indispensable: the storage ring’s cryostat, which houses the delicate superconducting coils that generate the experiment’s uniform magnetic field. “We want to measure nonuniformities in the vacuum. We don’t want to measure nonuniformities in the magnet,” explains Brendan Casey, a Fermilab physicist working on Muon $g-2$ ’s new detectors. “And if we didn’t have a perfect magnet, that’s all we would see.”

“There’s no other magnet like this anywhere,” adds Bill Morse, a Muon $g-2$ physicist at Brookhaven. To build another one at Fermilab would cost upwards of \$25 million—with no guarantee that its field would be as uniform as the original’s. Moving it, on the other hand, would cost only about \$3 million. It was decided: They’d transport the ring halfway across the country, without disturbing—much less dismantling—the cryostat and its delicate coils.

“When we first started talking about this years ago, we sort of naturally assumed you’d want to [move] it by helicopter” to a waiting barge, says Chris Polly, the project manager for Muon $g-2$. But unable to find a route through the Illinois suburbs that was devoid of people—a required safety precaution in case the ring were to unexpectedly plummet back to Earth—the scientists enlisted the help of Emmert International, a heavy haul transportation company best known for recently steering a 340-ton boulder destined for the Los Angeles County Museum of Art through L.A.’s streets. After months of feasibility studies, the team settled on a plan to transport the ring to and from a barge by truck, creeping along shuttered highways at 10 miles per hour in the dead of night so as not to disturb day-time traffic.

The superconducting coils cannot handle more than two millimeters of deflection, so throughout its journey, the cryostat will be held steady by a 40-ton transport fixture that will sit inside the ring and clamp onto it with eight spokes, freezing it in place like the tire on a bicycle wheel (see photo, below). “We’ve never had [a project] that is this delicate,” says Terry Emmert, executive vice president of Emmert International. The precision required is “unheard of in our industry.”

Although the St. Lawrence Seaway and the Great Lakes offered a slightly shorter passage to Chicago, the team decided to take the long way up the Mississippi and Illinois rivers to Lemont, Illinois. From there, a truck will pick up the final leg of the journey to the prairie laboratory. The southern route saves



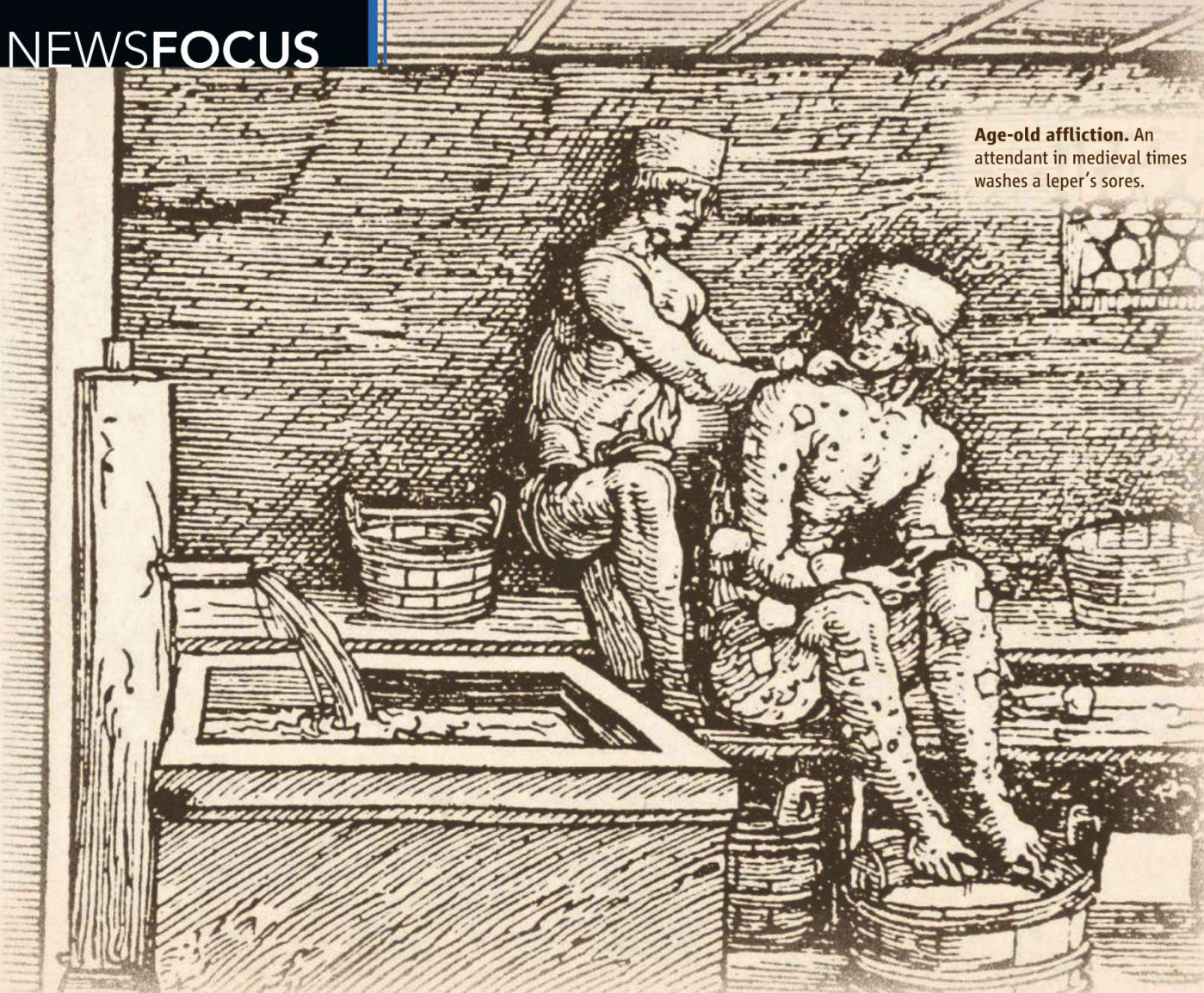
Circuitous. Researchers chose a long sea-and-river route for relocating a massive but delicate storage ring (below) from Brookhaven National Lab to Fermilab.

about \$300,000 and offers better weather—as long as the barge beats the worst of hurricane season. “We’ll be biting our nails for a few weeks,” Hertzog says.

Once the ring reaches Fermilab, physicists will spend the next year reassembling the experiment and outfitting it with new detectors. Then the researchers will fill it with liquid helium, test the coils, and find out at last whether their painstaking detour paid off.

—LIZZIE WADE

Age-old affliction. An attendant in medieval times washes a leper's sores.



On the Trail of Ancient Killers

Armed with new methods, researchers are interrogating the DNA of centuries-old pathogens extracted from the bones and teeth of victims

LAST AUGUST, JOHANNES KRAUSE WAS PERFORMING A FEAT THAT no one would have dared attempt a few years ago: He was carrying out a genetic autopsy on 700-year-old human remains. Working with DNA extracted from the tooth of a young woman who died in the 1300s in a leper colony in Denmark, Krause was calculating how much of the jumble of genetic material from microbes, contaminants, and humans was from the pathogen that caused the woman's leprosy, *Mycobacterium leprae*. He hoped for about 1% to 2%. To his surprise, a whopping 40% of the DNA aligned with the modern *M. leprae* sequence. Just 9% was human. *Impossible*, thought Krause, a paleogeneticist at the University of Tübingen in Germany.

He did the analysis again and got the same answer. "It was crazy," he says. "I never expected to find way more pathogen DNA than human DNA in a human bone. You'd never find that in a modern

patient who has leprosy."

Krause excitedly called his graduate student, biochemist Verena Schuenemann, who had extracted and prepared the bone powder from the tooth. They realized that they had hit a scientific jackpot. "With this high concentration of bacteria, we'd try to make a dream come true," Schuenemann says. They set out to directly sequence the genome of an ancient bacterium—a first for the field—rather than resorting to laborious techniques required in the past to capture degraded ancient DNA.

Now, online this week in *Science*, they and their colleagues unveil their success, publishing the complete genome of medieval *M. leprae* from direct shotgun sequencing (<http://scim.ag/VSchuenemann>). They were able to get 100 copies on average of each of the 3.3 million bases in the microbe's genome, far better than the 20 copies on average that is the standard for modern bacterial genomes.

CREDIT: SPL/SCIENCE SOURCE

“We could just reconstruct the genome from scratch,” Krause says. “It was like creating a reference genome from an ancient DNA source.”

The breakthrough is all the more amazing because researchers have yet to sequence the *M. leprae* genome directly from living humans with leprosy. The bacteria cannot be grown in cell culture, so researchers infect armadillos and mice with human *M. leprae* to get enough to sample for sequencing.

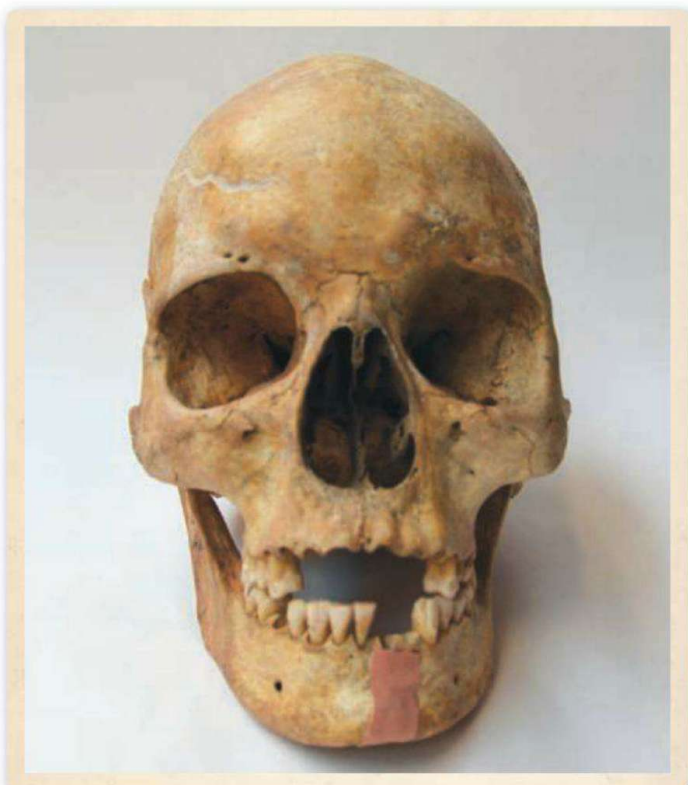
This technical triumph would have been unheard of 5 years ago. It reflects the turbo boost that the small but energized field of ancient DNA research has gotten from next-generation sequencing machines, invented for the human genome, plus clever new techniques to extract target DNA from a soup of ancient molecules. Krause’s team got lucky in their sample, but even those with more modest amounts of ancient DNA are succeeding at nabbing pathogens. “We’ve had incredible advances with genetic sequencing,” said Charles Nunn, an evolutionary geneticist at Harvard University, in April at a symposium on the evolution of infectious disease at the American Association of Physical Anthropologists meeting in Knoxville. “It’s critical for understanding the relationships between circulating pathogens today and those in our past.”

Awash in data, several labs are racing neck-and-neck to cull DNA from a Most Wanted list of legendary killers: tuberculosis (TB), plague, cholera, *Leishmania*, leprosy, the potato blight, and AIDS. They gather traces of these culprits from ancient teeth, bones, hair, feces, and—in the case of potato blight—from skin and leaves, then unleash the sequencers. The work, which began in earnest 3 years ago, adds a new dimension to our understanding of historical events, revealing the true nature of the villains responsible for humanity’s worst epidemics. “There are a lot of diseases described in the historical record that we don’t know what the pathogen is,” says molecular anthropologist Anne Stone of Arizona State University, Tempe.

The research is also illuminating where pathogens first infected humans and how they have become more virulent, or less, over time. For example, knowing if urbanization long ago triggered the evolution of nastier TB strains “could inform the best strategy for vaccination and drug treatment” in today’s increasingly urbanized world, says biomolecular archaeologist Terence Brown of the University of Manchester in the United Kingdom.

The problem with PCR

None of this was thought possible in the early 1990s, when Stone was a graduate student. She was trying to spot genetic markers for ancient smallpox and TB, working in the lab of Svante Pääbo at the Max Planck Institute in Munich (now in Leipzig). The research was challenging—pathogen genomes are 1000 times smaller than human genomes and can resemble those of garden-variety soil microbes. Back then, researchers were excited if they could sequence a handful of DNA markers from fossils, using the painstaking polymerase chain reaction (PCR).



Stunning find. A tooth from a 14th century Danish woman had more DNA from the leprosy microbe than from her own genome.

But Stone couldn’t even nab a few snippets. She tried to amplify the *Variola major* virus that causes smallpox from a 400-year-old mummy from Naples, Italy, but got nothing. She gave up on ancient pathogens and worked on ancient DNA from skeletal remains and the evolution of apes and humans.

As the years passed, other teams announced having used PCR to ferret out TB from skeletal remains, but critics worried that the evidence was contamination. Even when they could confirm ancient TB, PCR studies shed no light on how ancient TB strains are related to today’s *M. tuberculosis*, because they couldn’t get enough ancient DNA to reveal differences between strains. “I don’t care if someone says they got TB from a 15,000-year-old sample,” says evolutionary biologist Thomas Gilbert of the University of Copenhagen. “I want to know, what can you tell me about that TB?”

The advent almost a decade ago of high-throughput sequencing machines eventually revolutionized the field. While PCR can copy only DNA fragments that are at least 80 bases long, the new machines decode each base separately: a boon for ancient DNA, which is often recovered in fragments smaller than 80 base pairs. The sequencers opened a whole new world of ancient genomes, including sensational reports of DNA recovered from ice age mammoths, bears, and extinct humans such as Neandertals and Denisovans (*Science*, 26 August 2011, p. 1084).

That work drove the development of new “targeted enrichment” methods to extract more



Disease detective. Verena Schuenemann extracts ancient DNA from the remains of victims of the plague and other scourges.

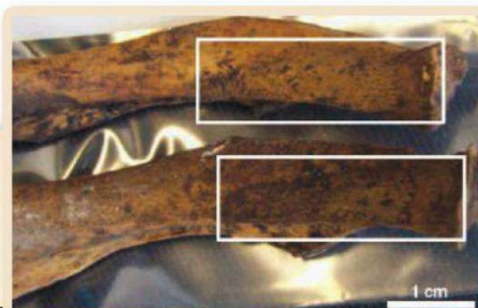
ancient DNA from precious fossils. With Pääbo, Krause used one such an approach to sequence the Neandertal genome (*Science*, 7 May 2010, p. 723). That got him thinking of scaling a new peak: DNA from old pathogens.

Fishing for genomes

No. 1 on the Most Wanted list was the agent of the Black Death, or bubonic plague, which wiped out up to half of Europe's population between 1347 and 1351. Historic documents described buboes—swelling in the groin and armpits—resembling those caused by the bacterium *Yersinia pestis*. Today, this bug infects a few thousand people a year in Africa and Asia and is endemic in prairie voles in the southwestern United States. Even before antibiotics, *Y. pestis* hardly seemed potent enough to claim millions of lives, so some historians doubted that it was the force behind the Black Death. Early PCR results from medieval victims found markers for part of a gene from modern *Y. pestis*, but the charges didn't stick: The results couldn't be replicated.

In 2011, researchers turned to targeted enrichment. Hendrik Poinar of McMaster University in Hamilton, Canada, working with Sharon DeWitte of the University of South Carolina and McMaster graduate student Kirsten Bos, extracted DNA from teeth and bones from the East Smithfield burial ground in London, where plague victims were buried in the 14th century. Working with Krause and Schuenemann, the two

Written in bone. DNA from TB lesions on a 19th century teenager's rib bones (right) revealed the strain that infected her. TB deformed this 13th century teenager's spine.



labs used “bait”—short DNA segments found in modern *Y. pestis*—as probes to fish out corresponding stretches of ancient sequence. The researchers rinsed away DNA that didn't bind to the modern bait. This let them snare not only matching sequences, but also DNA in flanking regions that might vary from the modern genome. By scouring overlapping DNA segments over and over, they reported in *Nature* in 2011, they gathered 30 copies on average of each nucleotide.

This outstanding resolution confirmed that the London plague victims were infected with *Y. pestis*. To the team's surprise, the genome of the 14th century *Y. pestis* was remarkably similar to the modern one—a puzzle because it suggests that the same bacterium was far more lethal in the 14th century than it is today. Now, the team is exploring whether the high mortality rate was caused, for instance,

by one of the few differences in the genetics of the ancient strain, or by the poor health of medieval victims.

Work by several groups on ancient and modern samples is laying bare the origin and evolution of the Black Death strain. Researchers assumed that the plague was caused by a new strain that reached Europe in 1347, soon after its emergence. But in November, Krause and colleagues reported in *PLOS ONE* that the Black Death strain originated in an earlier burst of diversification. By comparing plague DNA from ancient and modern collections worldwide, they found 11 strains circulating at the time of the Justinian Plague in Europe in the 6th to 8th century C.E., including the strain that later caused the Black Death.

This fits with another team's study of more than 100 strains of *Y. pestis* from modern humans and rodents in Asia. Yujun Cui of the Beijing Institute of Microbiology and Epidemiology and his colleagues used the modern samples to trace the origin of many strains, including the one that caused the Black Death, back to a “big bang” of diversity around the time of the Justinian Plague, as they reported last year in the *Proceedings of the National Academy of Sciences (PNAS)*. The analysis shows a starlike pattern of evolution, with a burst of new strains of *Y. pestis* appearing in short order.

Such a pattern is predicted by a population genetics model created by Francois Balloux of University College London. In his model, new strains of *Y. pestis* rapidly appeared during epidemics when humans on the move, such as Crusaders or other soldiers, spread the disease into new regions. There, the pathogens went on a tear in new hosts. When the Black Death waned in 1351, the culprit strain replicated less often and its mutations became “fixed,” or stable, which is why it is so similar to today's strain, Balloux says.

Detailed analysis and modeling of the ancient killer may clue researchers in to what's happening when a new pathogen emerges—and how to combat it. The technological advances have also spurred many young researchers to visit Krause's lab in Tübingen, at a rate of one every 2 weeks, to learn targeted enrichment. “It's important to share the methods to raise the quality to these new standards,” Krause says. “Otherwise, people would still publish papers with PCR evidence, like they've been doing the past 20 years.”

TB or not TB?

TB is the second deadliest infectious disease in the world, after HIV; in 2011, it killed 1.4 million people. In the past decade, researchers have revised their view that all human TB infections are caused by almost identical strains of *M. tuberculosis*. Now, they recognize that there are seven distinct groups of TB strains, with two found only in Africans or recent African immigrants (suggesting an African origin of the disease).

TB and humans go way back: The earliest archeological evidence comes from a 7800-year-old skeleton found in a cave in Liguria, which has tubercular damage to its spine. The earliest historical records date to 4700 B.C.E., in China. As a result of co-evolution with humans and other animals for many millennia, groups of strains have evolved separately in the Old World (Africa, Europe, and Asia) and in the New World (the Americas). Researchers are eager to understand how New World and Old World strains are related to each other and to animal strains and how the movements of infected humans altered TB's virulence. They'd also like to know whether drug resistance develops at

CREDITS: GEORGE ROBERT MILNER/ILLINOIS STATE MUSEUM; (INSET) A. BOUWMAN ET AL., PNAS 109, 45 (22 OCTOBER 2012)

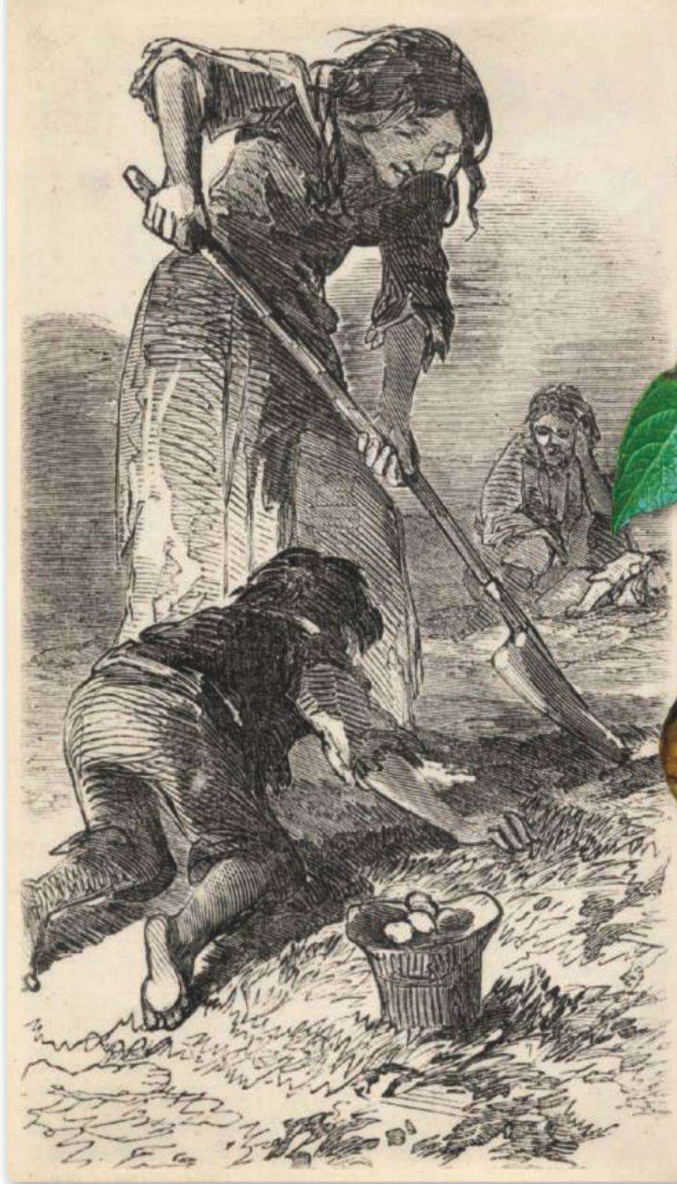
the same sites in different strains and how quickly.

Answers may lie in the depths of time. Stone and graduate student Luz-Andrea Pfister have already shot down the old notion that humans first acquired TB from cows; it turns out that we gave it to them (*Science*, 2 May 2008, p. 608). Recent work following the trail of modern strains back to a common ancestor suggests that TB may have been infecting hominids in Africa as long as 3 million years ago.

No one has yet sequenced a complete ancient *M. tuberculosis* genome, but researchers are making headway. Last year, a team sampled TB lesions on the rib of a teenage girl who was buried with her twin sister sometime between 1840 and 1911 in St. George's Crypt in Leeds. In a clean room at the University of Manchester, Brown and his former postdoc Abigail Bouwman, now at the University of Zurich, used next-generation sequencers and targeted enrichment, deploying 500 baits to fish out 218 single-nucleotide polymorphisms (SNPs) known to vary among modern TB strains. By analyzing the presence or absence of those SNPs in ancient and modern strains, they compiled the first set of DNA markers for a historic TB strain.

Comparing the historic strain with the same regions of all seven human strains and 10 animal strains, the team found that the girl from Leeds had a strain uncommon today but virtually identical to one isolated from a patient in New York in 1905. The finding vividly shows how pathogens could traverse great distances well before air travel. "There were a lot of people moving across the Atlantic—a lot of immigration to the U.S.," Brown says. It also shows that the strain was replaced by new ones in 100 years. Because the twin girls came from a family who could afford to bury them in a crypt, the find supports a long-held view, based on historical documents, that TB "was the scourge of the middle class" in the expanding cities, Brown says.

The complexities of TB in living people mean that data on ancient strains are extremely valuable, says molecular epidemiologist Sebastien Gagneux of the Swiss Tropical and Public Health Institute in Basel. He has proposed that TB has been co-evolving with humans and our ancestors for so long that people from different continents are more susceptible to particular strains. He reported in *PNAS* in 2006, for example, that U.S.-born patients of Chinese and Filipino ethnicity tend to harbor the same TB strains as patients born in China and the Philippines, respectively—and that it was harder for them to become infected with strains circulating in their adopted land. Gagneux is eager to find out where different TB strains evolved and if they continue to infect their his-



Famine starter. An extinct strain of a funguslike pathogen devastated Ireland's lumper potato crop in the mid-19th century.

toric hosts at a higher rate than other populations. If so, that would have "important implications for tuberculosis control and vaccine development,"

Gagneux says, because, for example, it could influence which strains are included in vaccines. Stone, meanwhile, is examining whether Paleo-Indians carried TB as they migrated from Asia to the New World.

Woes of the Irish

Between 1845 and 1852, more than a million people in Ireland perished when potato crops failed. A million more fled the impoverished land for America and other nations. The nation never fully recovered. Ireland's present population of 4.5 million is less than three-quarters of what it was at the start of the famine.

Many blame the famine on politics as well as botany—many tons of food were exported from Ireland to England while people starved—but there's no doubt that the potato blight, a funguslike pathogen called *Phytophthora infestans*, was the chief culprit. Ireland heavily relied on a single variety, the lumper potato, to feed its growing population, and once lumper potatoes became susceptible to the blight, people had few other varieties to fall back on. Even today, potato blights cost farmers worldwide about \$6.2 billion a year, on average.

Most researchers have blamed the Irish potato blight on a *P. infestans* strain called US-1, which is a direct descendant of a strain responsible for a blight outbreak in North America starting in 1843. This strain predominated worldwide until the late 1970s.

But it's not the strain that researchers found when they analyzed

dried leaves of potatoes and tomatoes collected from Europe and North America between 1845 and 1896 and stored at the Botanical State Collection, Munich, and the Royal Botanic Gardens, Kew. A team led by Kentaro Yoshida of The Sainsbury Laboratory in Norwich, U.K., and Schuenemann got a pleasant surprise when they sequenced DNA from these samples: As with the 700-year-old leprosy samples from Denmark, the potato blight pathogen was so well



Mystery solved. DNA of the leprosy strain infecting armadillos shows that we gave them the disease.

preserved that they could shotgun-sequence it directly. They reported last month in *eLife* that the ancient plants were infested with a strain of *P. infestans*, HERB-1, that is different from all 15 modern strains, including US-1, although it and HERB-1 are closely related. “An extinct strain caused the pandemic in Ireland and Europe,” says Krause, a co-author.

The common ancestor of these strains probably emerged in the Toluca Valley in Mexico, where diverse strains of *P. infestans* infect wild relatives of the potato, Krause says. HERB-1 presumably reached Ireland by stowing away on ships sailing from the Americas, he says.

Work in press in *Nature Communications* confirms and extends this result. Gilbert and colleagues sequenced five European strains of *P. infestans*, including one from a potato leaf collected in Belgium in 1845, during the first reported outbreak of the blight. Their samples suggest that the pathogen was introduced to Europe more than once, where it found a vulnerable host—lumpers—and took root.

The potato famine’s societal consequences included a flood of immigration from Ireland to the United States—and on this front, too, ancient DNA may soon have a tale to tell. One of the scourges that the Irish faced at the end of their overseas journey was cholera, which ran riot on the East Coast in 1849. Cholera, nicknamed the blue death, brings severe diarrhea and turns a victim’s skin blue-gray from loss of fluids. It was first detected in India in 1817, and researchers have long assumed that Irish immigrants introduced it to the United States. Poinar’s graduate student Alison Devault is testing that idea with samples of *Vibrio cholerae* from 19th century samples of intestines, kept in jars in the Mütter Museum of the College of Physicians of Philadelphia. The findings may yield clues to why the *Vi. cholerae* strain now devastating Haiti is so virulent.

Medieval scourge

Now that they have a high-resolution copy of the complete genome of *M. leprae*, Krause and colleagues have a chance to bore deeper into the dreaded disease, notorious since Biblical times. The disease ravages the skin, peripheral nerves in the hands and feet, and mucous membranes of the nose, throat, and eyes. Today, leprosy is nowhere near as prevalent as it was in medieval times. Just before 1300 C.E., 25% or more of the adult population in Scandinavia, for instance, died with skeletal signs of leprosy, according to molecular archaeologist Ben Krause-Kyora of the University of Kiel in Germany. Even though the disease can be cured with antibiotics, 225,000 new cases appear every year in developing nations, where disfigured patients are often still shunned and in some nations banished to leper colonies.

After Krause-Kyora sent the medieval leper victim’s tooth to Krause for analysis, they compared the ancient *M. leprae* genome with those of 11 modern strains and found that the bacterium has evolved very slowly. The pathogen from the medieval leper was almost identical to the modern strain that causes leprosy in India, Thailand, Brazil, and the United States.

The plague bacterium, *Y. pestis*, also shows evolutionary stasis since medieval times. But the process of spawning strains appears to differ in these two pathogens. Whereas *Y. pestis* seems to have spun off a cluster of new strains around 600 C.E., *M. leprae* has apparently continuously generated new strains, at a slower pace. *M. leprae*’s evolutionary diagram looks more like a classic phylogenetic tree, with new strains branching off on occasion, than the star pattern seen in *Y. pestis*, Bos says.

Krause’s team found that the medieval Scandinavian girl’s strain of leprosy probably diverged from a strain found in Iran and Turkey today, suggesting that the ancestral strain originated in Asia. Crusaders or other travelers may have carried it to Europe around 1000 C.E., where it eventually reached Scandinavia. Leprosy finally declined in the 16th century.

Yet it has persisted: Several million people live with it worldwide, including about 30 to 40 people in the southern United States who contract it every year. Until recently, no one knew how they got it. Then, in 2011, microbiologist Richard W. Truman of the Health Resources and Services Administration’s National Hansen’s Disease Program in Baton Rouge and his team sequenced leprosy genomes from armadillos, one of the few nonhuman species afflicted by the disease. The team found that 65% of human infections were a distinct TB strain found in most of the infected armadillos. This suggests that they are getting leprosy from armadillos, presumably from handling them. But did armadillos spread the disease to people, or did we give it to the armored mammals first? Schuenemann and Krause report that we’re to blame: The striking closeness of the armadillo leprosy strain to the medieval girl’s strain suggests it “is of European origin,” Krause says.

Finally, Krause has also found a solution to the mystery that initially baffled him—why was there far more *M. leprae* DNA than human DNA in an ancient human tooth? He realized that the *M. leprae* bacterium has a particularly thick cell membrane, rich in fatty acids that repel water and thus damage. If true for other bacteria, this suggests good prospects for tracing some pathogens back beyond the theoretical limit of human DNA resisting degradation: 1 million years. “This could allow us to study bacteria much father back in time,” Krause says. Last year, he was hoping for a modest amount of ancient DNA from a medieval tooth. Now, he’s imagining sequencing pathogens that plagued our ancestor, *Homo erectus*, 1 million years ago. In ancient DNA research, what a difference a year makes.

—ANN GIBBONS

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SOLID-EARTH SCIENCE

Geophysical Exploration Linking Deep Earth and Backyard Geology

Big Science came to solid-Earth studies when the \$400 million EarthScope program offered a sharper view of the interior that could help geologists; it's working, mostly

RALEIGH—Geologists tramping over the volcanic rocks of Montana's Yellowstone or the high Colorado Plateau in the 1960s had little patience with the geophysicists trumpeting what would soon be called plate tectonics. What could seafloor spreading at the bottom of the Atlantic Ocean possibly have to do with the maddeningly complicated jumble of rocks that is North America?, the continental geologists asked.

Soon enough, however, the geophysicists won them over. Plate tectonics did neatly explain some major geologic features, such as the Appalachian Mountains and the volcanoes of the Pacific Northwest. But 30 years after the plate tectonics revolution, geologists still weren't content. What about fiery Yellowstone in midcontinent, the cryptically elevated Colorado Plateau, or any number of other quirky geologic features that still weren't fitting into the big picture of plate tectonics?

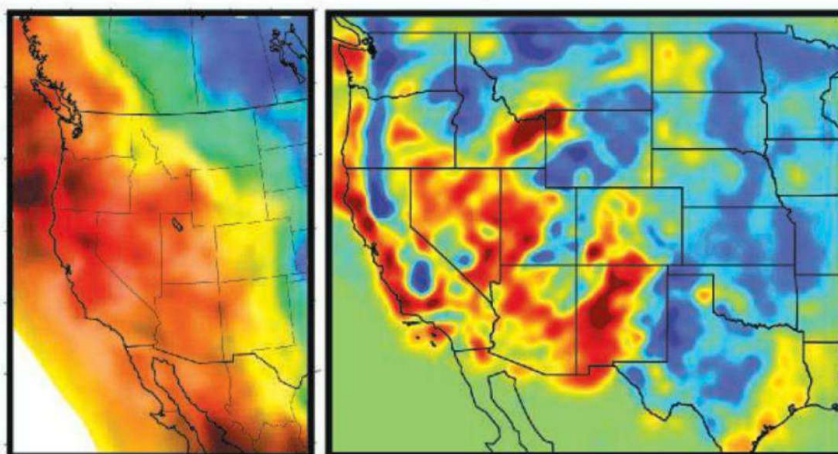
So around the turn of the century, geophysicists decided how they might finally satisfy the geologists. EarthScope, a proposed four-pronged program, would point a geophysical "telescope" inward to bring the subsurface of North America into unprecedentedly sharp focus. A wave of 400 seismometers would roll from coast to coast imaging the deep interior. A net of GPS instruments and a radar satellite would precisely gauge the grinding of plates along the West Coast from Mexico to Alaska. And a single borehole in central California would probe the heart of the threatening San Andreas fault.

No one could say just what these largely undirected explorations would discover, geophysicists conceded, but explanations for some of geologists' lingering mysteries would surely emerge as geophysicists

brought into focus deep details approaching the scale at which geologists work on the surface.

Ten years on, EarthScope is looking like a good investment. "The amount of very high-quality data and science generated by EarthScope is spectacular," says geophysicist Jean-Bernard Minster of the Scripps Institution of Oceanography (SIO) in San Diego, California. "EarthScope was the right idea. We've learned a lot more about Earth the last

Western United States at a Depth of 90 Kilometers



That's better. Seismic data from EarthScope's Transportable Array sharpened existing imaging (left) so that colder (blue) and hotter (red) features in the mantle stand out (right).

10 years than the previous 30 to 40 years. I can say that because I don't get any money from EarthScope."

Beneath North America, it turns out, creatures of the deep Earth roam. From a hot, rising, deep-rooted plume fueling Yellowstone to a cold falling "drip" helping unloose the Colorado Plateau, these deep features do indeed shape geology at the surface. Not that all was smooth sailing. Drilling into even tiny earthquakes proved particularly

challenging. But as the program's wave of traveling seismometers washes up on the East Coast, the pleasant surprises are far outweighing the shortfalls.

Online

sciencemag.org

Podcast interview with author Richard A. Kerr (http://scim.ag/pod_6138).

Going big science ...

From the start, EarthScope was unlike other projects built through the National Science Foundation's (NSF's) Major Research Equipment and Facilities Construction account. At \$197 million for construction over 5 years, it was certainly major. But compared with physicists building a gravity wave detector or astronomers an observatory, geoscientists have "a cultural core that is fundamentally different," geophysicist David Simpson, president of the Incorporated Research Institutions for Seismology in Washington, D.C., told the biennial EarthScope National Meeting here last month.

EarthScope's facility has not one but thousands of instruments of several sorts distributed coast to coast and from the Mexican border to Alaska. There are no EarthScope principal investigators in line for a Nobel Prize, only a community-based manage-

ment structure. EarthScope data are not held for analysis by a select few, but immediately available to anyone anywhere in the world. And its "scope" will not be scanning the interior for any particular Holy Grail discovery, just looking around.

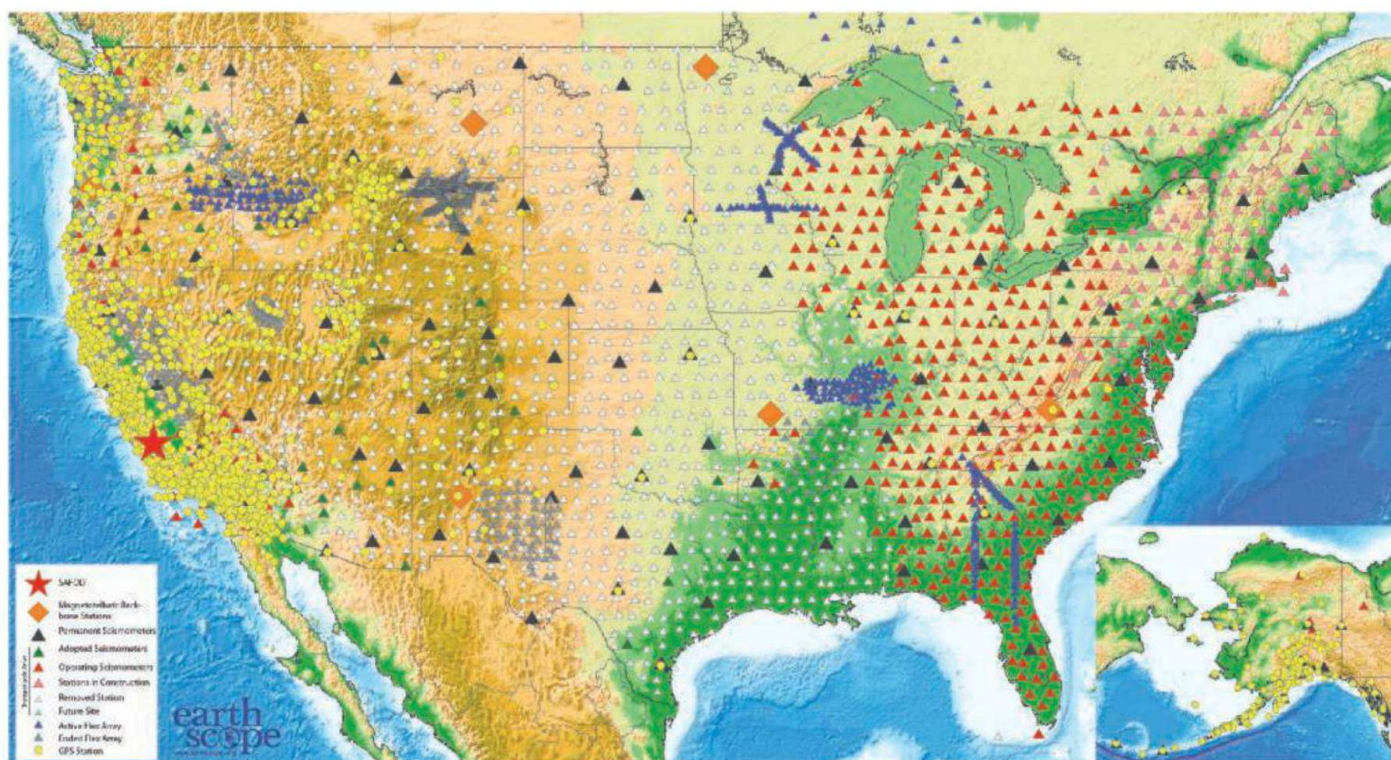
"If you have lots of good data," observed seismologist Edward Garnero of Arizona State University, Tempe, "discoveries flow." Those discoveries, researchers reason, will almost certainly help them understand the

North American continent and help predict earthquakes and volcanic eruptions as well.

To get the big picture

For EarthScope's deepest and broadest look into Earth, seismologists rolled out USArray: 500 seismometers to record seismic waves passing through and altered by the rock beneath North America. Plenty of other seismometers were already in operation, but most were bunched around dangerous, quake-producing faults in the West.

USArray (\$69 million for construction and \$88 million for operations so far) took a much broader view using more sophisticated instruments. Researchers spread 100 seismometers evenly across the lower 48 states in a regular grid with 300 kilometers between them. Those would remain where they were



Got it covered. The EarthScope program was intended to sharpen geophysicists' view of the interior by using more uniform and denser instrument networks. GPS stations (yellow circles) gauge building crustal strain. Seismometers

allow mantle imaging (triangles: black, green, and red are in use; pink are future and white past transportable sites; blue are in temporary local study networks). Red star is core-drilling site.

installed. But then, hard against the Pacific coast, geoscientists deployed 400 more seismometers in an 800-kilometer-wide swath between the Canadian and Mexican borders with 70-kilometer spacing.

Then they marched this 400-strong Transportable Array (TA) eastward, one station at a time. After a seismometer had been operating 2 years, field engineers would extract it from its half-buried vault on the trailing edge of the TA and reinstall it to the east on the leading edge, one instrument every day or two for 10 years until a 2000-strong instrument grid reaches the Atlantic coast; that should happen in September. Just as more pixels in an electronic camera yield a sharper image, the dense, continent-spanning TA grid would produce clearer views of the subsurface.

And it's working. "The things being seen are much smaller," Garnero says, by an order of magnitude. And the greater detail is showing that "the mantle is doing stuff." Seismic analyses can construct 3D images of the interior based on how hotter or colder rock slows or speeds up seismic waves from distant quakes. Earlier, fuzzier seismic images had already shown hotter-than-normal rock a few hundred kilometers beneath Yellowstone. But images constructed with the sharper TA data show that the hot rock extends through the upper mantle beneath the North Ameri-

can plate and into the lower mantle at least 900 kilometers down. That is the first rising, hot-rock plume that most seismologists can agree is rooted in the lower mantle (*Science*, 5 April, p. 22).

The Yellowstone plume most likely starts at the mantle's bottom, hard against the molten-iron core, but it obviously hasn't had an easy rise. As imaged by seismologist Brandon Schmandt of the University of New Mexico, Albuquerque, and his colleagues, the plume seems to have encountered resistance about 600 kilometers down that created a gap in the plume. The obstacle appears to have been a sheet of tectonic plate that dove into the mantle beneath the western edge of North America tens of millions of years ago and then broke apart. In another study using TA data, seismologist Eugene Humphreys of the University of Oregon in Eugene and his colleagues suggested that the sinking slab broke apart when a chunk of particularly thick descending plate jammed at the continent's edge about 50 million years ago (*Science*, 14 January 2011, p. 142).

Indeed, slabs of sunken plate can now be clearly seen dangling here and there around the western lower 48. Schmandt and others have pointed out that sinking slabs would have let hot mantle rock invade the shallow mantle until the hot, melting mantle rock came up

against the continental plate. TA's improved seismic images do seem to imply such local mantle upwellings. These often coincide in space and time with major episodes of volcanic outpourings such as the outbursts that formed the Idaho Batholith and the Columbia River flood basalt, suggesting a connection, researchers say.

And then there are the drips. These are the antiplumes, the bottommost layers of the continental plate that peel away or slough off and sink because they are colder and therefore denser than adjacent mantle. Relieved of the burden, the overlying plate can rise. One drip clearly seen in TA data probably let the southern Sierra Nevada float upward. Using TA data, seismologist Alan Levander of Rice University and colleagues have shown how drips off the bottom of the Colorado Plateau have helped let it rise to its 2000-meter altitude. The current drip has been falling away for the past 6 million years, they find. What with a rising plume, sinking drips, and melting mantle boring into the continent, Humphreys says, "we're remaking continents."

Probing the plate boundary

The rest of EarthScope took a broad but exceedingly shallow look at the western boundary of the North American plate, with a single, pinpoint boring into the San

Andreas. In addition to the seismic networks of USArray, the 2001 EarthScope Project Plan proposed two components that would focus on the zone from Mexico to Alaska where the Pacific plate grinds by the North American plate. Only one of the proposed components came to pass.

The Plate Boundary Observatory (PBO) component of EarthScope (\$100 million for construction and \$58 million for operations so far) consists of 1100 GPS instruments installed in a broad swath of the western United States. They continuously measure their changing position to about 1 millimeter per year. Over the long haul, the two plates are moving by each other at about 30 millimeters per year. But if the plates snag on the San Andreas or on any of the broad maze of faults spanning the plate boundary, the stress would squeeze or stretch the surrounding crust, moving the GPS instruments. All told, PBO GPS continuously records the building strain—or sudden release of strain in quakes—at points separated by tens to hundreds of kilometers.

Unlike GPS, the second planned geodetic component—a satellite-borne interferometric synthetic aperture radar (InSAR)—would have mapped changing strain everywhere across the plate boundary, not just at widely separated points. An InSAR satellite can gauge the motion of contiguous spots on the surface a few tens of meters across with a precision of a few millimeters. With such spatial continuity, geodesists could have been far more precise about gauging the strain building toward failure on a particular segment of fault, geodesist David Sandwell of SIO told the meeting.

But a U.S. InSAR satellite with a focus on the western plate boundary was not to be, at least not yet. Although NSF could chip in some seed funding, researchers had to sell NASA on the idea. Minster has been the principal investigator on four InSAR proposals, “none of which worked,” he says. “I’m not quite sure why.”

But even without InSAR, EarthScope’s PBO has been delivering. PBO observations have revealed the often complex release of quake energy along a fault. PBO strain data are also being fed into calculations of fault-by-fault earthquake hazards that will produce the next Uniform California Earth-

quake Rupture Forecast gauging risk.

And it turns out there’s more shakin’ than the San Andreas. In another bit of EarthScope serendipity, PBO observations have helped reveal a jiggling of the Great Basin of Utah and Nevada. The crust there is stretching east to west over millennia, but GPS is showing that over a few years, a part of the Great Basin can go from extending to contracting or the entire basin can shift to the east or west. Tectonophysicist Brian Wernicke of the California Institute of Technology in Pasadena and colleagues have argued that somewhere tens of kilometers beneath the surface, the rock above is able to slip across the rock below. That would mean the whole system could be responding to the churning of the

down 1.8 kilometers west of the fault and then angled it to intercept the fault at a depth of just over 3 kilometers. The plan was to punch out cores of rock across the fault and bring them to the surface, along with fault fluids, for analysis. Then workers would lower an instrument package down the hole to monitor fault conditions and to listen to the tiny quakes that pop off nearby.

Drilling through hard, broken rock is neither easy nor cheap, but SAFOD drillers managed to recover core from a weak section of the fault. Fifty years ago, geologists proposed that clay was lubricating the sides of the fault in such spots so the fault would not stick. Tectonophysicist David Lockner of the U.S. Geological Survey in Menlo Park, California, and colleagues confirmed that idea by showing that clay formed by alteration of the mineral serpentine had rendered the fault there slippery and “profoundly weak.”

SAFOD efforts to understand how and why other fault segments stick and then break did not work out so well. Drilling problems created extra costs, according to SAFOD co-leader Mark Zoback of Stanford University. On top of that, the cost of even routine drilling soared as the growing Chinese economy sucked up raw materials and an oil-drilling boom in North Dakota drove up the cost of leasing a drilling rig. So NSF money for drilling ran out before a nearby stuck patch of fault could be cored. Then a leaky seal wrecked the instrument package days after it was lowered into the hole.

Still, “overall, I view EarthScope as very successful,” says

geophysicist Seth Stein of Northwestern University in Evanston, Illinois, excluding the satellite component that was never attempted. He was among the half-dozen researchers who first took the idea for an inward-looking scope to NSF. “Two out of three components were on time and on budget and are doing everything they were supposed to. Two point five out of three ain’t bad.”

And it isn’t over. The USArray is only just now reaching the East Coast, and then it will be moved on to Alaska. For that matter, seismologists are still swamped by the volume of USArray data and are only beginning to look farther east and even deeper while applying more sophisticated analytical techniques. The mantle aquarium may soon have more inhabitants.

—RICHARD A. KERR



A deep grab. Workers extract a core from 3 kilometers down in the SAFOD drill hole into the San Andreas fault. The core showed clay weakening the fault.

mantle brought on by the disrupting sunken plate imaged by USArray.

A hole not far enough

The final EarthScope component took a far narrower view of North America: down a 15-centimeter-wide hole drilled next to the San Andreas in central California. Planners intended that the \$27 million San Andreas Fault Observatory at Depth (SAFOD) would help understand the chemical and physical processes that cause the opposing sides of the San Andreas to quietly slip by each other in some places and stick, build stress, and fail in an earthquake in other places. Those are good things to know if you’re wondering about the next Big One.

So SAFOD workers drilled a hole straight

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LETTERS

edited by Jennifer Sills

The Age of Man: A Father Figure

IN HIS NEWS & ANALYSIS STORY "ARCHAEOLOGISTS SAY THE 'Anthropocene' is here—but it began long ago" (19 April, p. 261), M. Balter reports that the "Age of Man," characterized by detrimental environmental changes caused by human activities, may have begun thousands of years ago. This hypothesis was proposed more than a century ago by Alfred Russel Wallace (1823–1913), one of the greatest evolutionary biologists of the 19th century (1). Wallace is well known as the codiscoverer of the Darwinian principle of natural selection and as the founder of biogeography (2).

As Wallace was interested in many subjects, including anthropology, psychology, politics, and economics (1), he was well qualified to evaluate the impact of humans on natural habitats from an evolutionary perspective. In 1898, he described "[t]he plunder of the earth," with reference to the "struggle for



Alfred Russel Wallace

wealth" by irresponsible humans (3). Wallace lamented the "reckless destruction of stored-up products of nature ... not equaled in amount during the whole preceding period of human history" and the "clearing of the (tropical) forests ... to make coffee plantations." He concluded that "[t]he devastation caused by the great despots of the Middle Ages and of antiquity ... has thus been reproduced in our times" (3).

In 1910, Wallace described the era of human environmental destructiveness, which started with the systematic use of fire and the possession of weapons for hunting (4). He also argued that "the extinction of so many large Mammalia (at the end of the Pleistocene) is actually due to man's agency" (4). Hence, Wallace is the spiritual father of the "overkill hypothesis"—i.e., the idea that extensive hunting by early humans may have caused megafaunal extinctions, which led to zoologically devastated ecosystems (4).

The year 2013 marks the centenary of Wallace's death. It should be acknowledged that this "unselfish man in the shadow of Darwin" (1, 2) was the first scientist who outlined, in his popular books (3, 4), what we today (unofficially) call the Anthropocene.

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The Age of Man:
Outpacing Evolution

IN THE NEWS & ANALYSIS STORY "Archaeologists say the 'Anthropocene' is here—but it began long ago" (M. Balter, 19 April, p. 261), Bruce Smith explains that the term "Anthropocene" was originally proposed as "a strategy for getting the public to appreciate the extent to which humans were destroying the world." In this case, one might define the beginning of the Anthropocene as the point in time at which humans begin altering the environment more quickly than biodiversity and ecosystems evolve and adapt to those changes. According to this definition, the era began long after the beginning of agriculture and even later than the beginning of

the Industrial Revolution in 1750 CE. We propose that the Anthropocene began in the United States in the 1930s and spread globally during the green revolution from the 1950s to the 1970s.

During this time period, the human appropriation of net primary production increased markedly (1), and the quantity of materials mobilized, either directly or indirectly, by humans (anthropogenic flows) began to exceed the corresponding flows of resources unaffected by humans (geogenic flows). Recent abrupt changes, such as the green revolution and China's rapid industrialization, differ substantially from the pace of previous changes, such as agricultural practices, which evolved over centuries and thus allowed species and species interactions to coevolve. The systems resulting

from those gradual changes maintained an equilibrium that benefits conservation, sustainable production, and cultural ecosystem services. To protect future human civilizations from the effects of the Anthropocene, we should work to decelerate change to gain time for evolution (2) and prevent breakdowns in ecosystem services (3).

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Shale-Gas Plans Threaten China's Water Resources

THE IMPACT OF SHALE-GAS DEVELOPMENT ON American water quality has received wide attention ("Impact of shale gas development on regional water quality," R. D. Vidic *et al.*, Review, 17 May, p. 826), but potential impacts of China's accelerating shale-gas exploration on the nation's water crisis have been largely ignored.

China has the world's largest shale-gas reserves, at 36 trillion m³ (1). The country has an ambitious plan to produce 6.5 billion m³ of shale gas by 2015 (2). Thirteen provinces have been selected as priority areas. However, seven of these provinces are already plagued by water shortages, with less than 2000 m³ available per person, less than one-quarter of the world average. Four of the thirteen provinces are in Southwest China, and two of those have recently experienced severe half-year droughts (3). Shale-gas extraction will compete for limited water resources with agricultural, industrial, and domestic sectors. Hydraulic fracturing (fracking), the most widely used extraction method in China, consumes large volumes of water mixed with a range of additives. Due to complex geological conditions, Chinese shale-gas wells each consume 10,000 to 24,000 m³ of water (4, 5). The target gas production of 1.5 billion m³ in Sichuan will require 171 million m³ of water, equal to 10.5% of the province's domestic water demand (6).

Some 10 to 90% of fracking fluids are returned to the surface (7). Inadequate treatment introduces heavy metals, acids, pesticides, and other hazardous materials to soil and aquatic environments (8). This will exacerbate China's polluted water environment (9, 10).

Exploitation of China's shale-gas reserves offers opportunities to satisfy the nation's growing energy demands and reduce carbon emissions, but careful management and legislation will be required to avoid shortages and pollution of already stretched water resources.

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CORRECTIONS AND CLARIFICATIONS

News Focus: "The numbers game" by M. Hvistendahl (31 May, p. 1037). The total for the grant given by the National Natural Science Foundation of China to the China Health and Retirement Longitudinal Study was incorrect. It should have been \$813,000, not \$813 million. The HTML and PDF versions online have been corrected.

Table of Contents: (17 May, p. 781). On page 783, the teaser for the Report by A. S. Gardner *et al.* was incorrect. The correct teaser is "The contribution of glaciers to sea level rise is nearly as much as that of the Greenland and Antarctic Ice Sheets combined." The HTML and PDF versions online have been corrected.

News Focus: "How big a role should neonicotinoids play in food security?" by E. Stokstad (10 May, p. 675). The credit for the chart was incomplete. The chart was adapted from: L. Maxim, J. van der Sluijs, in "Late lessons from early warnings: Science, precaution, innovation," European Environment Agency, Ed. (European Environment Agency, Report No. 1/2013, Copenhagen, 2013), ch. 16, p. 417. The HTML and PDF versions online have been corrected.

News & Analysis: "More high-tech visas, more STEM education funds" by D. Malakoff and J. Mervis (26 April, p. 415). The accompanying picture was mislabeled. The students in the Bridge 2 Engineering program at Saddleback College were visiting Terra Universal Inc. to learn about equipment used in critical environment applications. The label has been corrected in the HTML and PDF versions online.

Research Articles: "A massive pulsar in a compact relativistic binary" by J. Antoniadis *et al.* (26 April, p. 448). The Web site in reference 33 should be www.casina.virgo.infn.it. The HTML and PDF versions online have been corrected.

Policy Forum: "Right-sizing stem-rust research" by P. G. Pardey *et al.* (12 April, p. 147). A misplaced insertion of the words "pests and" introduced an error. The correct sentence follows: "A \$51.1 million annual R&D expenditure is equivalent to investing \$0.23 per hectare of wheat in 2009; by comparison, U.S. wheat farmers spent \$34.56 per hectare on seed in 2009." In addition, the last lines of the acknowledgment were dropped. It should read as follows: "Acknowledgments: The authors thank M. Carson, R. Singh, B. Steffenson, and Y. Chai for insights and C. Chan-Kang and M. Hallaway for research assistance. This paper was prepared for the HarvestChoice project with support from the BMGF, the University of Minnesota, and the Commonwealth Scientific and Industrial Research Organization (CSIRO)." The HTML and PDF versions online have been corrected.

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The Human Animal

IN HIS PERSPECTIVE "ANIMAL CONFORMISTS" (26 April, p. 437), F. B. M. de Waal asks "Do animals learn from each other in the same way as humans do?" Implying that humans occur in a category separate from animals may have unintended consequences.

First, the "humans and animals" paradigm undermines the unifying concept of all

biology—evolution. Under this paradigm, it seems coincidental that a trait would occur in both humans and dogs, for example, despite their common ancestry.

Second, the loose use of "humans and animals" handicaps future learning. Research in science education has shown that students are prone to a number of misconceptions, which are often integrated into their cognitive framework early, making them remarkably difficult to correct (1).

Third, our future as a species hinges on a worldview that places humans deep within the context of ecology. We consider ourselves superior to other animals due to our sharp intellect, complex social structure, and domination of most ecosystems and natural resources. This feeling of separateness from the other members of our ecosystems hinders efforts to promote conservation and sustainability.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

BIOGEOGRAPHY

Is India's Pride Actually African?

Craig Packer

The Assyrians drew modern lions, *Panthera leo*, from firsthand experience in ancient Mesopotamia; lions were familiar to the Egyptian pharaohs; the Old Testament describes lions near Jerusalem; Alexander the Great killed a lion in Greece. But lion portraits also appeared in countries where they never lived. Maned lions adorn the medieval crests of England and northern Europe and the art of dynastic China. Just because a nation's cultural tradition celebrated the lion doesn't mean it was a native species.

Which brings us to the current distribution of *Panthera leo*, now restricted to the continent of Africa—with the curious exception of the lions of Gir Forest in the Indian state of Gujarat. For nearly two centuries, biologists have labeled Gir lions as “Asiatic lions” and designated them as a subspecies, *P. l. persica*. Genetic tests from the 1980s seemed to confirm this classification, because they found exceptionally low variation in blood enzymes—as expected from a long history of inbreeding in a small population. But the Gir lions show no obvious morphological distinctions from African lions. Whereas recent DNA tests reveal clear differences between the Gir lions and lions from eastern and southern Africa, they cannot be distinguished from North African lions.

In *Exotic Aliens*, wildlife conservationist Valmik Thapar argues that the Gir lions are directly descended from African lions. They were brought to India, he posits, as part of an international trade in wild animals that was well established by ancient times. As may be familiar to western readers, Romans imported lions from North Africa to the Coliseum to battle gladiators and to consume the occasional Christian. In contrast, Eastern rulers like Alexander the Great gained considerable glory from being the only men courageous enough to kill a lion. Thus, the Persians apparently imported lions for the glory of the shah, as did the Indians for their rajahs and emperors.

In the late 20th century, South African game ranchers gained notoriety for releasing captive lions in confined areas where “canned

hunters” could always shoot their trophies. But there is nothing new under the sun. For centuries, shahs, rajahs, and emperors confronted their royal quarry in hunting parks or menageries: tigers, lions, cheetahs, and leopards were corralled and often drugged with opium so the great leaders (or their wives or children) could spear, stab, or shoot large, dangerous animals in comfort and safety.

Thapar suggests that African lions were imported over at least five centuries to restock the royal menageries, where they were eventually domesticated. Gujarat had long been a landing point on India's western coast and was thus a likely portal for importing exotic animals. The 19th-century rulers in Gujarat's Junagadh District were known to breed lions; Gir was their private hunting park. But the ancestors of these Gir lions may well have escaped from earlier menageries during periods of political upheaval.

Exotic Aliens

The Lion and the Cheetah in India

by Valmik Thapar, Romila Thapar, and Yusuf Ansari

Aleph, New Delhi, 2013. 304 pp. INR595, \$54.75, £23.99. ISBN 9789382277552.

Today, Gir lions are so tame that tourists watch them on foot. Until the 1980s, tour guides led goats into the forest and tethered them to stakes. The lions patiently waited, not feasting until the guides moved away. Compared to wild African lions, this forbearance is inconceivable.

When I visited Gir in 1995, my Indian hosts drove around until we found a cattleherder who pointed off to the side of the road,

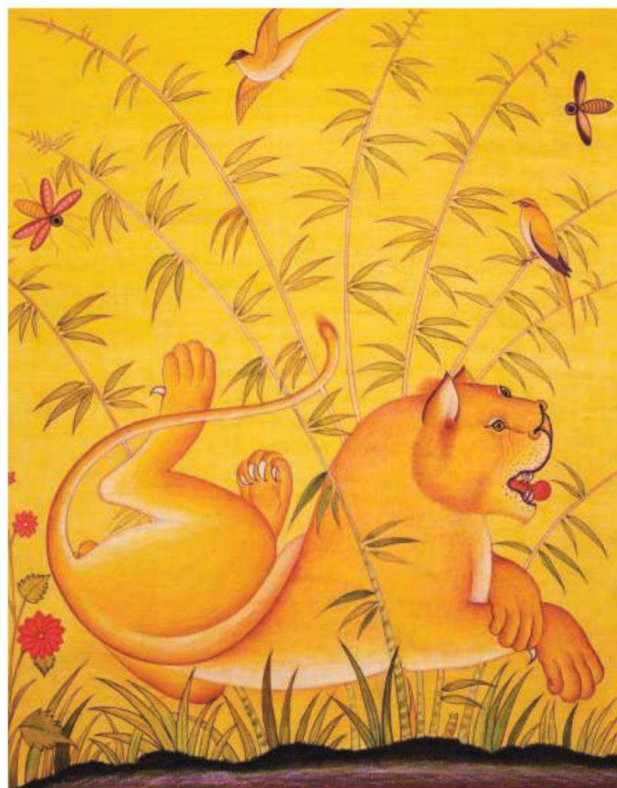
saying he'd just seen a mating lion pair. We left the car, and my hosts warned me not to walk too close. But I couldn't resist. I ducked beneath low branches, stepped over thick vines, and sat on the ground three meters from the pair. The female paid no mind, but the male reacted jealously. Then she rolled on her front and

started the low growling that invites the male to mount.

It was an intense moment. On the one hand, I was transported by the sensation of her low grumble vibrating through my fundament. But I also felt stupid for being so close at the impending moment of climax—when she would roll on her back and swat him in the face, whereupon he might charge at a third party. If she rolled counterclockwise, her swat would shove him toward me. But I was lucky. She rolled clockwise and pushed him in the opposite direction. He merely snarled at me.

Having been up close and nearly personal with Gir lions, I admit to being predisposed to accept Thapar's major conclusion: Gir's lions are odd, and it seems reasonable that their tameness derives from a long history of semidomestication. There's nothing physically or genetically distinctive about them; much of their diet consists of domestic livestock. Rather than having miraculously survived millennia of coexistence with people, maybe they do descend from escapees from a royal menagerie.

Thapar recruited the noted historian Romila Thapar and fellow historian Yusuf Ansari to write chapters highlighting the



Painting by Shaikh Usman Tirandaz based on Mughal art.

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absence of wild lions through the first few thousand years of India's recorded history. Lions never appeared in rock paintings or any other form of art until stone carvings in the third century BCE—carvings that look more like clumsy copies of Egyptian statues than the result of direct experience with lions. And whereas leopards and tigers were always clearly abundant in India, the virtual absence of lions century after century undermines the credibility that lions could have somehow persisted from prehistoric times.

India's Supreme Court recently ruled that Gujarat could no longer monopolize its lions, allowing the state of Madhya Pradesh to establish a second population in Kuno Sanctuary. This 18-year, ongoing dispute may seem trivial outside of India. But even if Gir lions descend from ancient menageries, they still hold political and cultural significance. Lions have persisted in Gir Forest for at least two hundred years in a country that is now home to a billion people. And the Gir lions may be living relics from a time when fierce creatures were brought to India, tamed, and drugged so royalty could perform repeated feats of "courage."

Exotic Aliens makes a persuasive case for reassessing the biology of these animals, and new genetic tests currently under way may more rigorously resolve the timing of the Gir lions' separation from their North African counterparts. Thapar further argues that cheetahs (*Acinonyx jubatus*) were also imported into India to populate the royal hunting parks and menageries. If he is correct, the current distributions of these charismatic felines in Asia are relicts of human actions rather than prehistoric ranges.

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PHYSICS

Quantum Illumination

Dan Browne

"I think I can safely say that nobody understands quantum mechanics" (1). With Richard Feynman's infamous quote, Christopher Gerry and Kimberley Bruno close *The Quantum Divide*.

Feynman's words have not deterred authors from writing about quantum mechanics. On the contrary, popular works on quantum physics often top the nonfiction bestseller

lists, and certain key textbooks on quantum mechanics have become a core part of undergraduate physics courses. Where then is there a gap in the market for another book on the quantum world?

Neither written for the general reader nor a textbook, *The Quantum Divide* instead sits halfway between these categories—technically detailed (it requires a certain mathematics and physics background) but conversationally styled (there are no derivations or exercises). The authors provide a concise introduction to the counterintuitive aspects of the quantum world through a tour of key experiments from the past three decades. Thought experiments help us capture essential physical concepts and think through to their consequences. Often used in teaching physics, they are particularly important in illustrating apparently paradoxical aspects of quantum mechanics. In the field's most famous example, Schrödinger's cat, the fate of the poor cat shows the apparent absurdity of applying quantum ideas to large-scale objects.

To many, however, a thought experiment—an experiment that may never be carried out—can seem far too abstract and can confuse as much as it illuminates. Technical advances have now brought these thought experiments into the laboratory. Gerry and Bruno introduce the quantum world through a curated tour of this recent research, laboratory realizations (largely with light) that have demonstrated many of the more provocative quantum thought experiments.

The authors begin, like many quantum texts do, with a chapter on the seemingly contradictory wave-like yet particle-like behavior of electrons. Unlike most treatments, however, their starting point is not the wave or the particle. Rather, it is the superposition properties of the quantum bit (or quantum coin, as they introduce it). A concept from quantum computing theory, the quantum bit is analogous to the bit, the basic unit of information in current classical computing. The basis of a quantum computer, a quantum bit is also the simplest possible quantum system. Gerry and Bruno use it to introduce the notion of quantum superposition, which, they explain, is the key property that leads to the wave-like behavior of particles such as electrons.

They then turn to photons, using photon experiments to illustrate key concepts such as interference, which-path information, and entanglement. Their chapter on quantum information provides brief introductions to quantum cryptography and

quantum teleportation, again focusing on early experimental realizations. They wrap up the tour of experiments with a discussion of Schrödinger cat-type experiments performed using superconducting devices. The concluding chapter addresses the problem of interpreting quantum mechanics, with a particular focus on the "quantum divide" of the book's title: how (or whether) the world can split into a quantum world obeying quantum laws and a classical world obeying classical laws.

I would recommend the book to current and former physics students as well as any mathematically confident, educated lay person having a high school background in physics. The authors do not shy away from using technical notation and mathematical ideas (such as complex numbers) that most lay readers would not feel comfortable with. The book would provide excellent supplemental reading for any undergraduate studying quantum mechanics. It will introduce them to key quantum concepts, such as entanglement, and beautiful (apparent) paradoxes, such as interaction-free measurement, that they are (sadly) likely not to encounter in their initial university lectures—often too focused on technical (albeit important) aspects such as solving Schrödinger's wave equation.

I particularly liked the early strong emphasis on the distinction between quantum superposition and classical randomness and the robust (while polite) debunking of some of the misleading ideas about quantum mechanics (e.g., particles being everywhere in the universe at the same time) often (if understandably) used by science popularizers. In places, the text is rather dense and ideas fly by rather too quickly. Perhaps the authors should have given more space to slightly fewer experiments.

Nevertheless, Gerry and Bruno succeed in introducing the quantum world in a readable but not oversimplified way. Their engaging and original account will particularly satisfy those who find popular texts on quantum mechanics lacking in technical detail. *The Quantum Divide* will leave readers understanding Feynman's quote with its original intent—not as an admission of defeat but as an invitation to the fascinating world of quantum physics.

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Why Schrödinger's Cat
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£25. ISBN 9780199666560.

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HUMAN RIGHTS

A Human Right to Science

Audrey Chapman^{1*} and Jessica Wyndham²

Although recognized in international law for decades, the right to enjoy the benefits of scientific progress and its applications has received little attention from the human rights and scientific communities (1–5). In 2012, Farida Shaheed, a Special Rapporteur (SR) for the United Nations (UN), presented a report to the UN Human Rights Council (HRC) on the meaning and application of the right (6). The next step is for the UN Committee on Economic, Social and Cultural Rights (CESCR) to adopt an official statement on the meaning and application of the right that provides clear guidance on the steps governments must take for implementation. The CESCR has been reluctant because of the complexity of issues and the need to know more about government practice. To address such knowledge gaps, the scientific and human rights communities need to provide input.

First internationally recognized in the Universal Declaration of Human Rights (1948), the right to enjoy the benefits of scientific progress is enshrined in Article 15 of the International Covenant on Economic, Social and Cultural Rights (1966) (ICESCR) (7). Addressing science through a human rights lens is both novel and potentially significant. Science policy guided by human rights principles may set priorities and allocate resources differently from policies driven by commercial interests, the interests of scientists, or national scientific competitiveness. Furthermore, addressing science through a human rights lens offers the potential to identify universal standards and norms that can facilitate “harmonizing global science” (8).

The SR report identifies four core components of the right: (i) access by everyone, without discrimination, to the benefits of science and its applications, including scientific knowledge; (ii) opportunities for all to contribute to the scientific enterprise and the freedom indispensable for scientific research; (iii) participation of individuals and communities in decision-making about science and the related right to information; and (iv) development of an enabling environment fostering the conservation, development, and diffusion of

science and technology (S&T).

For Article 15 to offer a unified and coherent vision for science as a human right, certain conceptual questions must be answered: What kind of infrastructure and policies are required to implement the right? What means of outreach and education would best enable public engagement in decision-making about S&T?

The SR emphasized that innovations essential to living with dignity should be accessible to all. This translates, according to the human rights community, into the need for budgets and products that address priority needs of

marginalized and poor populations, e.g., medicines for neglected tropical diseases, electricity for remote rural populations, potable water, and adequate sanitation. In conjunction with other obligations in the ICESCR, particularly Article 2, an obligation is presumed on the part of more-developed countries to furnish technical assistance in S&T and to enable access across borders to essential knowledge and technologies. The scope of assistance and technology transfer requires elaboration.

Article 15 is the nexus between the right to benefit from science and the right of creators to benefit, “morally and materially,” from their creations. In that context, the CESCR has emphasized the need for intellectual property regimes to be consistent with human rights commitments (9). How can this balance be achieved?

In addition, Article 15 directs states to recognize the benefits of international contacts and cooperation in scientific and cultural fields. It is disturbing that many governments are increasingly blocking or filtering Internet content and restricting academic freedom (10). It is also a concern that national security justifications are given for sometimes overzealous and unnecessary restrictions on the freedom of scientists to collaborate internationally (11). How can the international scientific community counter these challenges?

Lack of policy protections and oversight of S&T developments can pose challenges to human rights. For example, the U.S. Presiden-

The scientific community must inform UN efforts to promote universal access to the scientific enterprise and to benefits that ensue.

tial Commission for the Study of Bioethical Issues recently recognized that a U.S. study in Guatemala in the 1940s violated human rights by exposing vulnerable populations to sexually transmitted diseases (12). Often, one group benefits from technologies while others bear the brunt of risks and indirect costs (13). How can both equity and scientific progress be maximized?

The SR report recommended that interested groups contribute to defining the right. The American Association for the Advancement of Science (AAAS, which publishes *Science*) Science and Human Rights Coalition

Science policy guided by human rights principles may set priorities and allocate resources differently from policies driven by commercial interests, the interests of scientists, or national scientific competitiveness.

(<http://shr.aaas.org/coalition>) has been leading a process to elicit perspectives of scientists and engineers on the meaning of the right, barriers to its implementation, and steps governments can take to ensure realization of the right in practice. Findings are to be presented to the UN at the end of 2013.

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Dark Clouds over Spanish Science

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Spain's controversial 2013–2020 research and development (R&D) strategy (1), and the 2013–2016 national R&D plan that implements it (2) aim to (i) slim down public support for basic science and education and move it toward market-driven, applied research and (ii) foster private participation in technology transfer by redirecting public funds toward private enterprises. The plan's performance indicators focus more on technology transfer and innovation than on research quality (2). Improved private R&D would be welcome, but redirecting public funds toward private, target-based R&D, coupled with major budget cuts (3), and without addressing underlying issues in the Spanish production and funding systems, risks undermining efforts to sustain and improve basic research.

Private sector innovation in Spain is hampered by the small size and low innovative culture of most firms, the small high-tech sector and marginal growth of emerging sectors, the lack of financial system culture for funding innovation, the lack of collaboration between firms, and insufficient internal demand to drive innovation (4). About 59% of public R&D funding in Spain in 2012 was allocated to loans. But insufficient take-up from the private sector contributed to large portions of these funds being unused, making actual public research spending at least €3.01 billion less than allocated in the parliamentary budget (5).

Instead of drastically diverting public funds to market-driven research and to a private sector that may be ill-positioned to utilize the funds, the Spanish plan would benefit from a more balanced distribution of public investments in research and innovation and improved monitoring and evaluation of public investments in the private sector (in terms of knowledge generation and economic output). More efficient systems of incentives to attract larger investment of private funds



could build on successful examples of public-private partnerships. Such elements appear in the European Union's Horizon 2020 scientific strategy for 2013–2020 (6).

In the Spanish innovation system, mismatch between commercial or societal needs and academic research, the lack of critical mass, and the small number of academic spin-offs from technology-focused firms make it difficult for knowledge to circulate, which hampers multiplier effects (4). Financial restrictions and cuts in R&D expenditure may asphyxiate fragile synergies between private and public sectors, rather than foster competitiveness by pruning less productive elements. This situation is not unique to Spain; other European countries face the challenge of sustaining growth through innovation amid the economic crisis (7).

To be certain, there remains a critical need to build on successes and improve Spain's public basic-research enterprise as well (4), by reducing bureaucratic red tape needed to obtain and spend R&D funds, providing institutional support for internationalization, and addressing unequal distribution of R&D expenditure among regions (8). Evaluation systems must be improved to link funding to results, given the lack of meritocracy of its human resources system [dominated by cronyism, the lack of performance-based incentives and penalties for tenured staff, and the absence of codes of scientific integrity and policies to handle misconduct (4)]. Such policies could boost R&D quality and mitigate the drain of young talent away from Spain.

Spanish R&D policy should capitalize on the levels of basic research excellence built during the last decade.

As emerging economies increase investment in R&D (9), their scale and speed will be hard for Spain to match. Reversing budget cuts and optimizing how funds are used are critical. Basic research can translate into new technologies and have greater social and economic long-term impact than market-oriented research (10); excessive emphasis by governments and universities on agenda-driven research can jeopardize those benefits. Spanish policy-makers should capitalize on human and structural resources built during the last decade, as effective innovation only comes when applied research builds on foundations of basic science.

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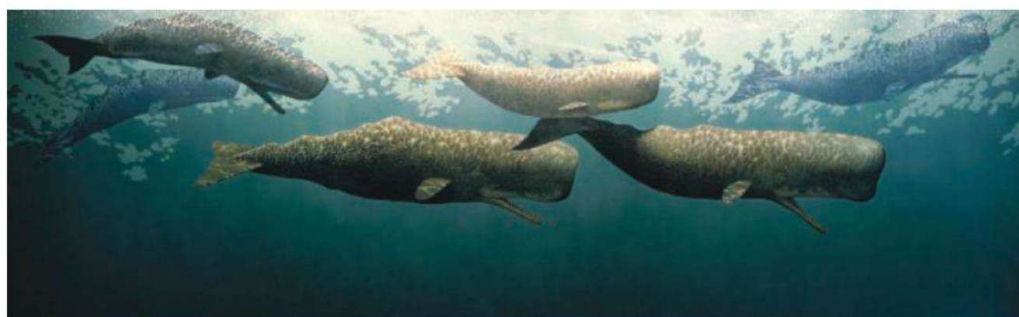
EVOLUTION

Better Oxygen Delivery

Enrico L. Rezende^{1,2}

The ability to use oxygen as the terminal acceptor of electrons in the cellular respiratory chain—a process that is substantially more efficient in generating energy from organic molecules than anaerobic alternatives—was a major evolutionary innovation that may have opened the doors for multicellular complexity (1, 2). At a given time in evolution, aerobic metabolism took the stage. Three studies in this issue, by Mirceta *et al.* (3) on page 1303, Natarajan *et al.* (4) on page 1324, and Rummer *et al.* (5) on page 1327, discuss how myoglobin and hemoglobin, respiratory pigments responsible for oxygen uptake, transport, and storage have evolved in vertebrates to meet the demands of different lifestyles.

Mirceta *et al.* traced how myoglobin, an iron- and oxygen-binding protein found in the muscles of vertebrates, has evolved across different mammalian lineages. The authors show that diving mammals (see the figure) acquired increasingly charged myoglobin to accommodate larger amounts of protein in their muscles. This change optimized myoglobin as an oxygen storage facility—the electrostatically charged proteins repel each other and are thereby less prone to aggregate. By becoming more soluble in the cellular milieu, charged myoglobin and the oxygen it carries is more abundant and readily available. By contrast, Natarajan *et al.* studied the oxygen affinity of hemoglobin variants in deer mice. Hemoglobin is another iron-containing protein that binds to oxygen and is responsible for its transport in the blood. In most vertebrates, hemoglobin consists of four subunits (two alpha and two beta). The authors demonstrate that the forms of hemoglobin prevalent in mice that inhabit high and low altitudes constitute two out of many possible combinations of these polymorphic subunits. The remaining combinations are poor oxygen carriers, likely removed from populations by natural selection. Hence, the adaptive value of any given biochemical substitution depends on the altitude and the genetic background of the local population. Rummer *et al.* describe how a highly acid-sensitive type of



Extreme physiology. Elite mammalian divers, such as the whale, can forage under water for long periods of time because of their enhanced oxygen storage capacity, acquired through evolved forms of myoglobin with increased solubility. This maximizes myoglobin concentration and oxygen reserves in muscles.

hemoglobin, present only in ray-finned fishes, and responsible for the so-called Root effect, can unload oxygen to peripheral tissues without compromising its uptake in the gills during blood acidosis. The Root effect describes highly specialized situations in which lower pH causes a decrease in hemoglobin's carrying capacity for oxygen. In fish, the effect allows hemoglobin to release oxygen into the swim bladder, which provides buoyancy and reduces the necessity to expend energy on swimming. The circumstances under which this response might have evolved have puzzled physiologists for decades.

Conceptually, these three studies represent nuances of a common theme—an association between aerobic capacity and high performance. Diving mammals rely on oxygen stores to sustain the activity of muscles while hunting prey under water. Increased oxygen stores would enable these lineages to exploit new food resources and avoid competition, accounting for the general pattern described by Mirceta *et al.* Similarly, efficient oxygenation of peripheral tissues allows deer mice to remain warm and active during harsh winters (6); therefore, their hemoglobin variants adapted to different altitudes might partly explain their abundance and widespread distribution, as suggested by Natarajan *et al.* The finding by Rummer *et al.* that oxygen delivery to the muscles is enhanced by the Root effect in the rainbow trout could imply that selection on swimming performance may have contributed to the evolution of this response in ray-finned fishes. Well-oxygenated muscles allow for sustained swimming at lower speeds and for the oxidation of lactate produced during sprinting, which would

The evolution of proficient oxygen delivery is associated with the rise of lifestyles that require enhanced performance.

prove advantageous for lineages that actively search and capture prey (7).

As proposed for birds and mammals (8), selection for enhanced oxygen delivery may have been associated with the adoption of increasingly active lifestyles in fishes. By relying on aerobic metabolism to maintain elevated activity levels, vertebrate lineages have seemingly employed the same general solution across land and water to deal with similar ecological problems. Furthermore, efficient oxygen-delivery systems have paved the way for the origin of evolutionary novelties considered essential for the success of these lineages: the ability to regulate, by physiological means, buoyancy in ray-finned fishes (9) and body temperature in birds and mammals (10).

These commonalities provide a general idea of how highly integrated complex physiological systems evolve. Ecological processes, such as competition or predator avoidance, drive the evolution of physiological attributes that enhance performance, which, in turn, may give rise to new ecological opportunities. The transition from land to water or across different altitudes has altered the selective regimes underlying oxygen usage and, as illustrated by Mirceta *et al.* and Natarajan *et al.*, the molecular structure of relevant respiratory pigments. From the opposite perspective, the function ascribed by Rummer *et al.* to the Root effect might clarify which selective pressures may have shaped this response in ancestral ray-finned fishes. Interestingly, selection for locomotor capacity may be particularly strong among visual fish predators (11); hence, the concomitant role of the Root effect for retina and muscle oxygenation might be more than a coincidence.

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The association between enhanced oxygen delivery and increased performance seems to be a recurrent theme, and illustrates how a mechanistic understanding of extant organisms can provide insight into the past. Nonetheless, sloths and koalas remind us that selection often favors slower lifestyles, and that highly derived forms may be inadequate to understand the factors that have shaped the evolution of their ancestors (12). In the absence of direct physiological and ecological evidence from the fossil record, establishing associations between different

lifestyles and various aspects of performance in ancestral lineages remains a major challenge. Only by integrating multiple lines of evidence, from broad comparative studies to detailed molecular analyses, may we understand how and why increased aerobic capacity has evolved repeatedly, and how it might have contributed to the evolutionary diversification of very disparate lineages.

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BIOCHEMISTRY

Watch Water Flow

Jeff Abramson^{1,2} and Armand S. Vartanian¹

Cells have numerous channels and transporters that facilitate movement of specific molecules and ions across biological membranes. However, it is unclear how these proteins facilitate the rapid passage of specific ions while impeding other, often very similar, substrates. The mechanism by which potassium ions (K^+) are transported through K^+ channels was revealed more than a decade ago (1–4). Computer simulations mirrored the x-ray data, demonstrating the complementary nature of the two techniques. On page 1346 of this issue, Kosinska Eriksson *et al.* (5) report the crystal structure of yeast aquaporin1 at subangstrom resolution in conjunction with molecular dynamics simulations, revealing that, much like K^+ in K^+ channels, water flows through the aquaporin channel in a pairwise manner.

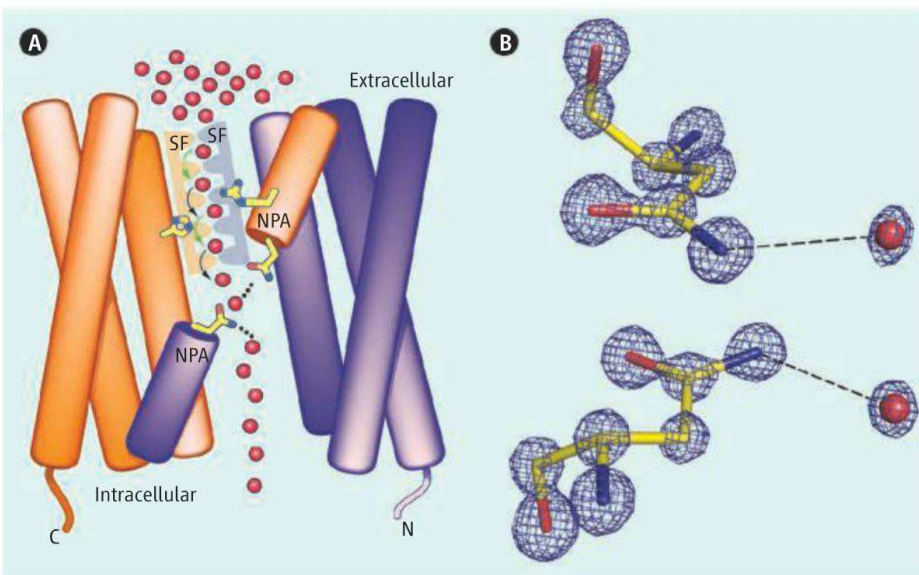
Aquaporins selectively and rapidly conduct water across biological membranes in response to osmotic pressure changes. Crystal structures have been determined for many aquaporins from a wide range of organisms (6–9). All structures show a conserved fold composed of six transmembrane α helices arranged in a homotetramer, with each monomer acting as an independent water channel. Aquaporins have an inverted repeat structure, where the amino- and carboxyl-terminal halves of the protein share a similar structural fold and are related by a twofold symmetry (see the figure, panel A) (10, 11).

In addition to the six transmembrane α helices, a seventh pseudo-transmembrane α helix is formed by reentrant loops from opposite sides of the membrane. Two conserved regions are responsible for selectivity (see the figure, panel A): the NPA-signature motif (asparagine-proline-alanine) in the center of the channel and the selectivity filter (SF) on the extracellular side, directly above the NPA-signature motif. These two elements orient

A high-resolution structure elucidates how water flows through aquaporin into and out of biological cells.

the water molecules through a narrow pore, where water selectivity is conferred by electrostatic and steric factors (12, 13). Together, these segments allow rapid water transport while excluding protons and other ions, thereby preserving the cell's electrochemical membrane potential.

A wealth of structural, mutational, and simulation data have driven various hypotheses regarding water translocation and the



High-precision water transport. (A) Aquaporins contain two conserved segments responsible for selectivity: the NPA signature motif (Asn¹¹² and Asn²²⁴, shown as ball-and-stick in the center of the channel), and the selectivity filter (SF) on the extracellular side of the channel. These two elements guide the water molecules (red circles) through a narrow pore, where selectivity for water is conferred by electrostatic and steric factors. Kosinska Eriksson *et al.* (5) show that water permeates through the pore in a choreographed pairwise fashion (black and green arrows), and that SF residues His¹¹² and Arg²²⁷ (shown as ball-and-stick on the extracellular side) exclude OH⁻ and H₃O⁺, respectively. (B) High-resolution electron density maps (5) show the precise hydrogen-bonding network between the NPA residues Asn¹¹² and Asn²²⁴ and two independent water molecules. Electron density is seen to be delocalized across the carbon-oxygen double bond.

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ability of aquaporins to exclude protons (13). Water molecules move in single file past the NPA and SF segments, where they are guided by hydrogen bonds from essential residues that mediate their movement while at the same time preventing the transport of hydroxide (OH⁻) and hydronium (H₃O⁺) ions.

Kosinska Eriksson *et al.* resolved the structure of yeast aquaporin 1 at ultrahigh resolution (0.88 Å, a record for membrane proteins) and in doing so could experimentally observe previously unresolved aspects of water transport. Many aquaporin structures have been solved at high resolution (<2 Å) (7–9), but the precise hydrogen bonding between key amino acids and the water molecules emerges only at subangstrom resolution. This unprecedented level of detail for a membrane protein provides a foundation for future studies to investigate the interplay between protein structure and water flux.

Aquaporins are highly selective for water transport while restricting proton flux. It has been proposed that a central water molecule simultaneously receives hydrogen bonds from both asparagine residues of the NPA, thereby preventing proton transmission via the Grotthuss mechanism, in which protons hop along a hydrogen-bond network of water molecules (13). In contrast, the new structure (5) reveals that the NPA asparagines donate hydrogen bonds to two independent water molecules (see the figure, panel B) while the bipolar distribution on the two halves of the channel is maintained. The study provides

a molecular mechanism for strongly constrained water conformational dynamics in both the NPA and SF regions, with the SF segment providing a more effective barrier for disrupting Grotthuss proton transport.

The new structure also helps to explain how the SF excludes OH⁻ and H₃O⁺ ions. Both the side chain of His²¹² and the neighboring backbone hydroxyl of Ala²²¹ simultaneously accept hydrogen bonds from a water molecule, enabling its passage. The passage of OH⁻, which can form only one hydrogen bond at this site, is thus precluded. Similarly, a localized positive charge on the side chain of Arg²²⁷ is concentrated in close proximity to the water pathway. This enacts a repulsive electrostatic effect upon any H₃O⁺ ions that may attempt passage. These observations explain why mutations to His²¹² and Arg²²⁷ impair the molecule's ion exclusion abilities.

Perhaps the most rewarding structural observation is the electron density for four water molecules located between His²¹² and Arg²²⁷ within the SF. Because of their close proximity to one another, all four water molecules cannot be bound simultaneously. Proposing a mechanism analogous to the pairwise movement of K⁺ ions through the SF of the K⁺ channel (3–5), Kosinska Eriksson *et al.* suggest that a pair of water molecules—separated by a vacant position—shift in choreographed fashion between two configurations (see the figure, panel A). As in K⁺ channels, the four water positions maintain almost equal crystallographic occupancy,

implying that the four sites are equally favorable, which allows for rapid transport through the channel.

The Kosinska Eriksson *et al.* study reveals an extremely specific and previously unknown water structure within the NPA and the SF segments of aquaporin. The similarities between the SF mechanisms in aquaporins and K⁺ channels may be indicative of convergent evolution, whereby these two protein families have ensured the efficient exclusion of nonsubstrate molecules while maintaining rapid flux across cell membranes. These critical experimental insights will bolster new theoretical developments and further drive mechanistic probing at an extraordinary level of accuracy.

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NEUROSCIENCE

Circuit Logic of Avoidance and Attraction

Chih-Ying Su and John R. Carlson

How an animal responds to a sensory stimulus depends on its intensity. Animals prefer food with moderate concentrations of salt and avoid food high in salt content. Responses may also depend on the context. Carbon dioxide (CO₂) emitted by stressed fruit flies elicits an avoidance response (1). However, CO₂ emitted by fermenting fruits is tolerated by flies in the context of food odors (2). How is a sensory stimulus encoded when presented at different

intensities or in different contexts? On pages 1338 and 1334 in this issue, Lin *et al.* (3) and Zhang *et al.* (4) provide new insight into this fascinating problem by investigating how different concentrations of CO₂ and salt are encoded by the olfactory and gustatory systems, respectively, in the fruit fly *Drosophila melanogaster*.

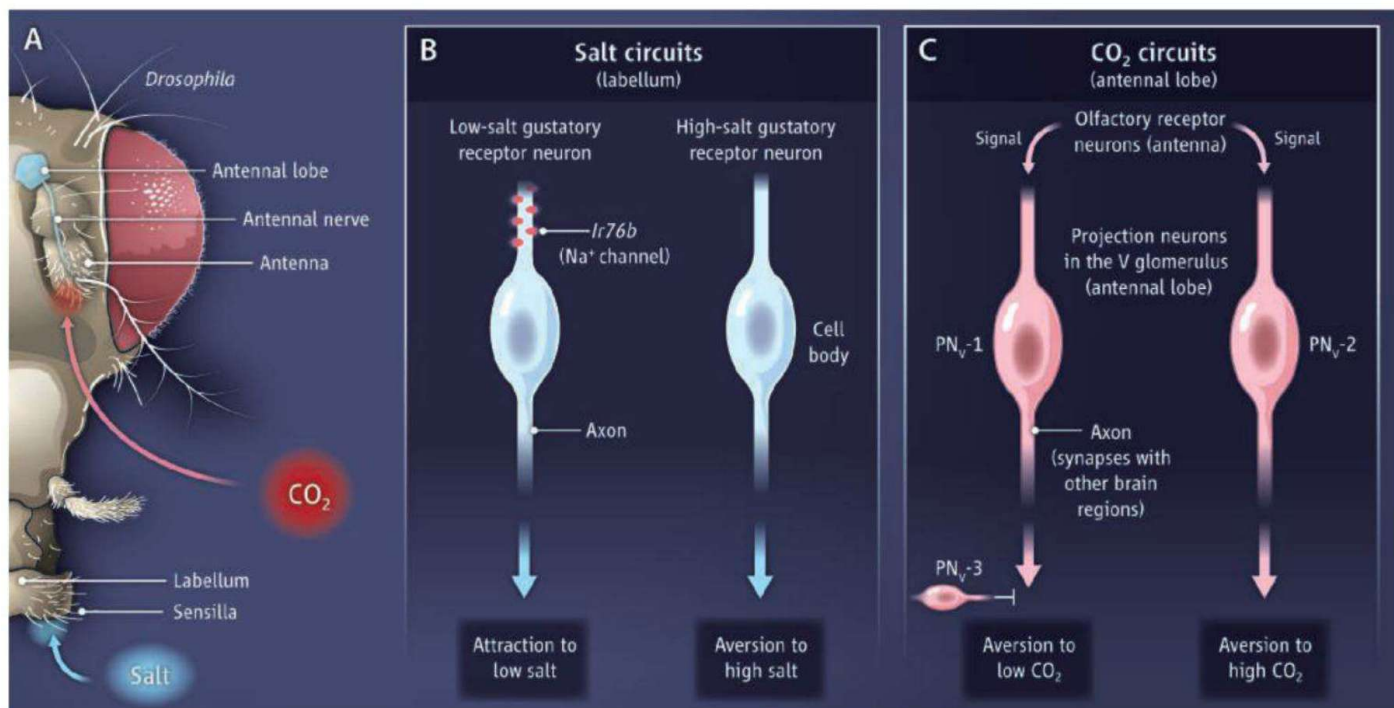
In fruit flies, the ab1C class of olfactory receptor neurons detects CO₂ (5). Activation of the ab1C neural circuit can drive robust aversion responses (1, 6). Yet CO₂ emitted by fruit does not cause aversion. Certain food odors may directly inhibit the CO₂ receptor of ab1C neurons (7). Alternatively, strong

The intensities and context of sensory stimuli are encoded by specific neural circuits that instruct behavioral responses.

activation of a neighboring olfactory receptor neuron (ab1A) by food odors may attenuate the response of an ab1C neuron through nonsynaptic inhibition (8). Both of these mechanisms operate in the antennae. Lin *et al.* provide another explanation for how low concentrations of CO₂ can be tolerated in the context of food.

From antennae, ab1C neurons send axons to a spheroidal module of neuropil (density of neuronal and glial processes) called the V glomerulus in the antennal lobe of the brain (see the figure). In the V glomerulus, the ab1C axon terminals form synapses with projection neurons. Lin *et al.* found that multi-

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Intensities and circuits. (A) Some components of the *Drosophila* sensory apparatus are shown. (B) Gustatory receptor neurons that mediate attraction to moderate salt levels (~100 mM NaCl) express the ionotropic receptor encoded by *Ir76b*. Neurons that respond strongly to high salt concentrations (>200 mM NaCl) medi-

ate aversion, which does not require *Ir76b*. (C) CO₂-responsive olfactory receptor neurons (ab1C) converge on the V glomerulus, where they relay information to projection neurons (PNs). PN_v-1 and PN_v-2 mediate aversion to low and high CO₂, respectively. Aversion to low CO₂ is inhibited by a fruit odor-responsive PN, PN_v-3.

ple types of projection neurons innervate the glomerulus and project to a variety of brain regions. Two types of projection neurons, PN_v-1 and PN_v-2, were found to be largely responsible for behavioral aversion to low (0.5%) and high (2%) concentrations of CO₂, respectively. As assessed by imaging intracellular Ca²⁺ (which reflects neuron activity), the response of PN_v-1 saturates at low concentrations of CO₂, whereas PN_v-2 responds more strongly at high concentrations of CO₂.

Why might signals from one sensory neuron type (ab1C) be channeled into two pathways (PN_v-1 and PN_v-2), both eliciting aversion to CO₂ but over different concentration ranges? One possible answer is that different concentrations of CO₂ may have different meaning to the fly and thus drive different behaviors. Another answer is that by segregating input into a low- or a high-CO₂ pathway, the two pathways can be differentially modulated. Indeed, Lin *et al.* determined that the low-CO₂ pathway, mediated by PN_v-1 can be inhibited by yet another projection neuron type, PN_v-3. Unlike most projection neurons, PN_v-3 innervates many glomeruli in the brain, including those activated by food odors. Thus, food odors activate PN_v-3, which in turn inhibits the PN_v-1 pathway. However, the PN_v-2 pathway appears uninhibited by PN_v-3. Consequently, food odors may block the PN_v-1-mediated aversion response to low

CO₂ concentrations present in a food source, while the fly retains its ability to respond to high CO₂ concentrations. High CO₂ concentrations also activate PN_v-3, which may inhibit the low-CO₂ pathway and accentuate the functional segregation between the low- and high-CO₂ pathways.

Is this circuit logic in the V glomerulus also used in other glomeruli? The ab1C circuit is unusual in that it is immune to pre-synaptic inhibition (9), a well-documented mechanism of central processing (9, 10). Two cardinal features of PN_v-3—its innervation of many glomeruli and its production of the inhibitory neurotransmitter GABA—could have arisen to provide an alternative mechanism for regulating the ab1C circuit. That said, the segregation of olfactory input into high- and low-intensity pathways that can be differentially regulated offers advantages that may have been used in other glomeruli.

Low and high concentrations of salt are also encoded by different neuronal pathways in the *Drosophila* gustatory system. Two classes of gustatory receptor neurons respond to salt with different sensitivities (11). They are located in sensilla of the labellum, the primary taste sensory organ in the head. Each sensillum contains multiple gustatory receptor neurons that respond to bitter, sweet, and salty stimuli. One class of these

neurons responds most strongly to moderate salt concentrations and drives an appetitive response; another class responds to salt most strongly at high concentrations and drives an aversive response. Zhang *et al.* show that *Ir76b*, a member of the ionotropic receptor gene family, is required for both the response of the low-salt neuron and the behavioral response that it drives. Moreover, ectopic expression of *Ir76b* confers sensitivity to low salt concentrations. By contrast, *Ir76b* is not required for physiological or behavioral responses to high salt. The authors propose that *Ir76b* encodes a channel that allows the influx of sodium into gustatory receptor neurons. Taken together, these findings highlight the importance of *Ir76b* in coding appetitive salt taste in *Drosophila*.

The studies of Lin *et al.* and Zhang *et al.* illustrate how a given stimulus, presented at different intensities, can activate different neural circuits in the fly. A similar principle applies in mammalian salt taste: Salt stimuli of low and high intensity elicit appetitive and aversive responses, respectively, through different circuits (12, 13). Much remains to be learned, however, about the fly circuits. The implications of the CO₂ circuit logic may be further clarified by a better understanding of the concentrations of CO₂ that the fly encounters in different contexts in its native environment. The salt results

invite further investigation of *Ir76b*, which is also expressed in a variety of cells that have not been previously implicated in salt taste.

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PHYSICS

Cold-Atom Magnetism

J. V. Porto

How do you cool something that is already the coldest stuff in the universe? That is the challenge facing physicists who are trying to use extremely dilute ultracold atomic gases to model condensed matter systems. Cold gases hold promise for simulating a variety of many-body problems, but progress has been limited by the inability to reach sufficiently low temperatures. On page 1307 of this issue, Greif *et al.* (1) show that by dynamically manipulating the atom-trapping potential, a gas of ultracold potassium atoms could be cooled enough to observe magnetic correlations that arise only at sufficiently low temperatures. These results mark an important step toward realizing quantum magnetism with cold atoms, an achievement that could lead to a better understanding of a range of interesting states of matter.

Since the observation that ultracold atoms in optical lattices represent a nearly perfect realization of the iconic Bose-Hubbard model of interacting particles in a lattice (2), there has been rapid growth in the study of many-body physics with cold, trapped atoms. At first glance, it may seem unlikely that a weakly interacting “material” that is 10,000 times less dense than air could resemble a condensed matter material at all. Although cold-atom densities and energy scales are certainly much smaller than in real solids, this is often merely a difference in scale. In many cases, the physics is essentially the same, requiring only that the gas be brought to an appropriately low temperature where the correlated physics of interest occurs. Because atomic gases can be cooled to low temperatures, they provide an attractive avenue for studying “condensed matter” physics in a controlled, nearly defect-free environment.

Starting with the realization of a Mott-insulating state (3), this approach has been successfully applied to a variety of problems (4).

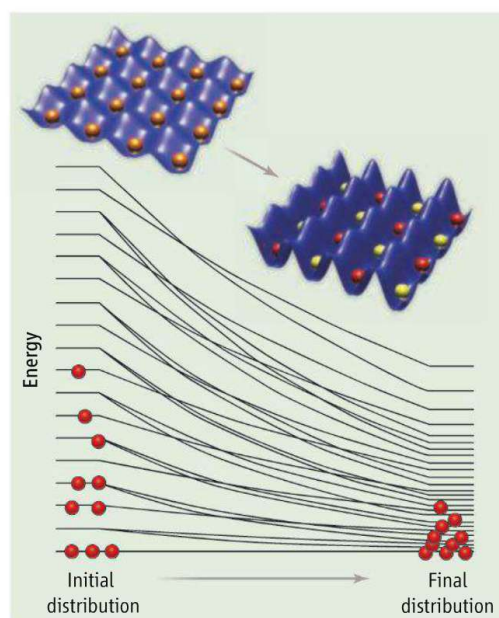
Unfortunately, many of the more interesting problems in condensed matter physics, particularly involving exotic states arising from quantum magnetism, require temperatures even lower than those achievable with current techniques. Based on exchange interactions between atoms in neighboring sites (5), the energy scales for many proposed cold-atom realizations of magnetism are much smaller than the typical onsite interac-

A trapped cloud of cold atoms can be used to simulate magnetism found in condensed matter systems.

tion energies. Despite the direct analogy with condensed matter systems, this cooling problem is complicated by a practical difference with real solids: It is very difficult to have an independent refrigerant in contact with a cold, dilute gas. New techniques for cooling are therefore needed (6).

One proposed solution is to use a separate cold-atom gas as a refrigerant (7), but this can be difficult to implement. Another approach is to dynamically manipulate the energy spectrum of the isolated system to reduce its temperature (8, 9). This approach has a direct analogy with adiabatic spin demagnetization cooling, where decreasing the internal energy scale of thermally isolated spins lowers the overall energy scale and leads to cooling. The key observation is that, whereas the second law of thermodynamics states that isolated systems cannot lower their entropy, there is no such restriction on the temperature if the internal energy spectrum of the system is changed. Such spin-demagnetization has been used to cool atoms in optical lattices (10), and entropy redistribution effects occur in large spin systems (11) and in nearly defect-free cold-atom crystals (12, 13).

Greif *et al.* use a similar approach in two-dimensional (2D) systems with magnetic interactions (5). Using a dynamically controlled multiperiod lattice, they raise the lattice potential along a subset of the lattice bonds. This changes the overall density of states of the system, thereby lowering the temperature. By raising the lattice along bonds that tend to isolate pairs of dimerized sites, they observe thermally equilibrated magnetic correlations within the dimerized sites. The resulting state is similar to spin-correlated states constructed by direct spin



Adiabatic cooling. Atoms are initially cooled as far as possible with conventional methods in a 2D isotropic lattice. Black lines indicate schematically the density of states, and the initial thermal distribution is indicated with red dots on the left. Raising the lattice depth along one direction isolates individual 1D systems and compresses the density of states, particularly for degrees of freedom associated with the now deep-lattice direction. The resulting thermal distribution is compressed (cooled) with respect to the degrees of freedom along the 1D tubes, giving rise to the observed magnetic correlations.

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manipulation (14), except that here the correlations arise thermodynamically.

In the second groundbreaking experiment, they demonstrate correlations in extended 1D systems. By raising the lattice along one direction, they convert the 2D lattice into an array of 1D systems (see the figure). The isolated 1D systems are sufficiently cooled to demonstrate antiferromagnetic spin correlations between neighboring spins. Remarkably, the correlations are observed to be symmetric, independent of which of a given spin's two neighbors are measured. This indicates that the adiabatically generated correlations extend beyond just two sites, a major achievement for cold-atom realization of magnetism.

The work here opens the door for a new set of challenges. Such low temperatures require careful understanding and control of all heating rates. This in itself can be an interesting area of research, because the mechanisms for heating can depend in detail on the many-body states (15). Also, because the adiabatic approach requires subsystems in which to dump entropy, it is not clear how far the technique can be pushed, or the best way to create cooling in other 2D lattice geometries. Nonetheless, the demonstration by Greif *et al.* of magnetic correlations in an extended cold-atom system provides optimism that these challenges can be overcome.

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10.1126/science.1239873

PHYSICS

Two Two-Dimensional Materials Are Better than One

Joachim M. Hamm and Ortwin Hess

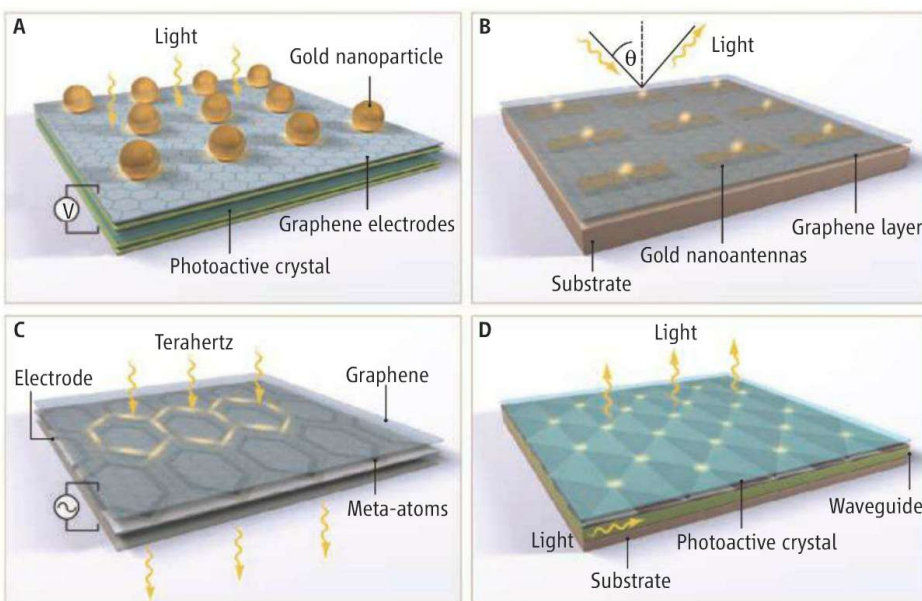
Extraordinary electronic or optical properties can result when layered solids are realized as two-dimensional (2D) materials (single or few-layer sheets), as is the case when graphene is formed from graphite. Optical properties can also be enhanced by restructuring materials at sub-wavelength scales into metamaterials, such as enhancing the plasmonic properties of gold—the coupling of light to electrons—by forming nanoparticles. Combining these approaches can lead to devices with capabilities that are otherwise difficult to realize. For example, for photovoltaic devices or sensors, materials with high electronic conductivity could be optically thick (to efficiently absorb light) but dimensionally thin (to impart flexibility and light weight). On page 1311 of this issue, Britnell *et al.* (1) combined highly conductive graphene and optically active 2D transition metal dichalcogenides into a heterostructure that photoexcites electron-hole pairs within a band-gap material. These carriers were separated with a p-n junction and extracted as a photocurrent with transparent graphene electrodes (graphene), and the performance was enhanced with plasmonic gold nanoparticles.

How does the light-matter interaction become stronger by making a particu-

lar material to become 2D, e.g., by exfoliation of single layers and making it so thin that it effectively has no thickness relative to the wavelength of light? This surprising property is directly related to the presence

Combining 2D materials and 2D metasurfaces enables the fabrication of photonic devices based on extreme interactions between electrons and light.

of critical points that generate in 2D or 1D (but not in 3D) the so-called Van Hove singularities in the electronic structure. Britnell *et al.* report that for the photoactive transition metal dichalcogenides such as molybdenum



Uniting flat materials. Various ultraflat photonic devices that could be built through the combination of 2D electronic materials and 2D photonic metasurfaces are illustrated. (A) Ultrathin broadband photovoltaic devices could be realized by combining 2D materials and broadband light harvesting (1). (B) Label-free single-molecule detectors could be achieved by exploiting a diffractive coupling between plasmonic nanoantennas functionalized by a graphene layer (10). (C) A polarization-independent terahertz modulator could be made by using gate-tunable graphene embedded in a metamaterial structure (11). (D) Ultrafast and broadband metasurface emitters could be made by tailoring the 2D active material to operate in visible light or in the terahertz regime (8, 12).

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disulfide (MoS₂), these singularities occur at visible frequencies and enhance the electronic density of states, i.e., the number of electrons per volume that participate in the absorption process at a given energy. Using this fundamental concept, a remarkably high quantum efficiency of up to 30% was achieved experimentally, in part by adding layers of hexagonal boron nitrate to provide homogenization of the doping levels.

The light-matter interaction was further increased by manipulating the local optical density of states. Britnell *et al.* show that depositing gold nanoparticles on the surface of the 2D heterostructure further enhanced the photogeneration of electron-hole pairs by a factor of 10. The nanoplasmonic particles form a broadband light-harvesting metamaterial (2) that concentrates field energy (or bundles optical modes) in “hotspots” under the particles. Additional generation of carri-

ers takes place where these hotspots overlap with the photoactive layer.

These results not only demonstrate that the simultaneous design of the electronic and the optical density of states is the key to an extreme control of light-matter interaction but also provide a glimpse of how future nanophotonic devices can benefit from the combination of two 2D materials: heterostructures of 2D atomic crystals (“electronic metamaterials”) to control the electronic wave functions and nanostructured metasurfaces (3–5) to control the light field. This principle, which we call the “United Flatlands” (6, 7) opens up many opportunities for the next generation of active 2D metamaterials (8) with quantum gain (9) and ultraflat photonic devices, such as solar cells, light-emitting diodes, nanolasers, and optical sensors. The figure showcases some of the most striking possibilities. With the potential to break many performance and

size limitations of bulk materials, particularly with respect to speed, energy efficiency, and area-footprint, the marriage of electronic and optical 2D (meta-) materials heralds a quantum leap in photonic device technology.

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10.1126/science.1239501

CELL BIOLOGY

Rapid Aging Rescue?

Thomas E. Johnson

Hutchinson-Gilford progeria syndrome (HGPS) is a rare dominant genetic disorder that mimics premature, rapid aging. Patients fail to thrive, and death occurs at an average age of 13 years, usually from myocardial infarction or stroke. Spontaneous HGPS-generating mutations occur at the rate of about 1 in 4 to 8 million births. *LMNA*, the gene harboring the HGPS mutations, encodes prelamin A (1). This precursor matures to lamin A, a structural component of the nuclear envelope. On page 1330 of this issue, Ibrahim *et al.* (2) show that methylated prelamin A, an intermediate form in the protein's maturation pathway, is associated with progeria symptoms in a mouse model of the disease. The methylated form blocks a signaling pathway that promotes cell proliferation, survival, and tissue growth. Reducing this methylation pharmacologically could be an effective therapeutic approach for treating progeroid disorders.

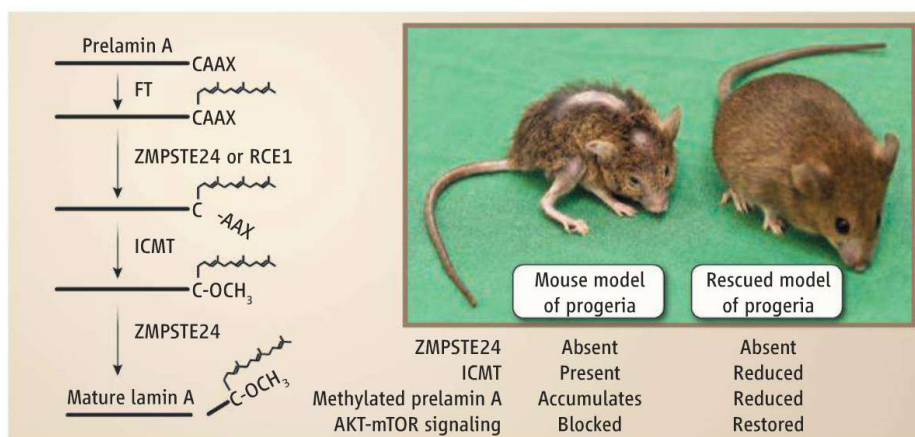
LMNA generates four peptides as the result of alternative splicing of its corresponding messenger RNA (mRNA). The two major protein products are prelamins A and C. Lamin A is a 664-amino acid protein that results from a variety of chemical and enzym-

atic processes (see the figure). The addition of a farnesyl group to prelamin A by farnesyltransferase is followed by removal of three amino acids from the distal end of the protein by either Ras-converting enzyme (RCE1) or the zinc metallopeptidase STE24 (ZMP-STE24) (3). Methylation of the remaining terminal cysteine is carried out by isoprenylcysteine carboxyl methyltransferase (ICMT), and a final clipping of the protein by ZMP-

Reducing methylation of the protein lamin A may provide a therapeutic target for Hutchinson-Gilford progeria syndrome.

STE24 to generate mature lamin A. The final cleavage step is altered in HGPS, producing a truncated form of prelamin A, often referred to as progerin.

Elucidating the synthesis of lamin A has an intriguing history. Initially, a mutation that causes HGPS was localized to chromosome 1q by genetic mapping. Three different mutations were then identified in 23 individuals diagnosed with the disorder (1, 4).



Signs of aging, reversed. Prelamin A is processed to lamin A, a constituent of the nuclear membrane. Mice lacking the gene encoding ZMPSTE24 show signs of accelerated aging that are characteristic of the Hutchinson-Gilford progeria syndrome. If there is reduced expression of ICMT as well in these mice, many aspects of aging are reversed. The rescued mice produced less methylated prelamin A and showed an increase in growth promoting signaling (the AKT-mTOR pathway). FT, farnesyltransferase; mTOR, mammalian target of rapamycin.

Each individual was heterozygous for one de novo mutation in *LMNA*, meaning that these were new mutations (not found in the parents) that likely arose from a single nucleotide base change (1, 5). However, the most common single-base mutation did not change the encoded residue. One would expect such a mutation to be silent (failing to have a mutant phenotype) rather than give rise to the severe symptoms of HGPS (1). It seems that the single-base change gives rise to a splice site within exon 11, resulting in the removal of 150 nucleotides from the corresponding mRNA. Consequently, this generates a 50-amino acid deletion near the carboxyl terminus of prelamin A (4, 5).

The 50 amino acids that are removed by altered splicing to exon 11 include the recognition sequence for ZMPSTE24. Mice lacking the *Zmpste24* gene present many of the characteristics associated with HGPS including bone fractures, muscle weakness, and a prelamin A processing defect (6). Ibrahim *et al.* noted that in this mouse model of HGPS, farnesylated and methylated prelamin A accumulated in the nuclear envelope. The authors found that decreasing the efficiency of terminal methylation of prelamin A in this mouse model, resulting from the expression of a hypomorphic allele of *Icmt*, reversed progeria-like symptoms, with increased body weight, a decrease in bone fractures, and a reduction in early death. The double-mutant mice showed a restoration of normal aging, instead of the senescence associated with additional rapid cell death seen in the *Zmpste24*-null mouse. The failure of fibroblast cells in *Zmpste24*-null mice to proliferate was also suppressed by reduced expression of ICMT. Thus, of all the aspect of aging studied, mouse longevity, grip strength, and cell senescence were changed to a more normal phenotype by reducing the amount of methylated prelamin A.

Surprisingly, Ibrahim *et al.* found that the frequency of misshapen nuclei—considered a hallmark of progeroid cells—in fibroblasts carrying mutations in both *Zmpste24* and *Icmt* was not different from that of nuclei in cells lacking only *Zmpste24*. However, localization of prelamin A was altered in the double-mutant mice; it localized to the nucleoplasm rather than the nuclear envelope in hepatocytes and skeletal muscle cells. Thus, the nuclear shape abnormalities appear to have little relevance to the disease.

Although the precise mechanism by which ICMT activity exacerbates the progeroid phenotype has yet to be determined, Ibrahim *et al.* show that decreasing the amount of methylated prelamin A in the rescued mouse

model of HGPS is associated with increased signaling by the AKT-mTOR pathway. This pathway promotes cell growth, proliferation, and survival. By decreasing the amount of methylated prelamin A in the rescued (double-mutant) mouse, AKT-mTOR signaling increased. The authors demonstrate interaction between prelamin A and AKT, but it is not clear if this association inhibits AKT-mTOR signaling.

In 2007, a clinical trial was launched at Boston Children's Hospital, in which 25 children aged 3 to 15 years received a farnesyltransferase inhibitor called lonafarnib for 2.5 years (7–10). The results showed modest but encouraging success that has been interpreted with caution. In 2009, another clinical trial was initiated with 45 children taking lonafarnib and two additional drugs, pravastatin and zoledronate (7). This combination may provide a more effective treatment than farnesyltransferase inhibitors alone, as pravastatin and zoledronate target different points in the lamin A maturation pathway (production or attachment of the farnesyl group). These two drugs themselves improved the phenotype of progeria cells and extended life span in mouse models of progeria (7).

That reducing isoprenylcysteine methylation pharmacologically may be helpful for

children affected by HGPS is an important implication of the basic research by Ibrahim *et al.* However, treatments for this disorder are not likely to be seen soon in the medicine cabinet of most 80-year-olds, as HGPS does not cause rapid aging but mimics it (11). There is some evidence that progerin is produced in the body with normal aging. For example, progerin has been found in aging blood vessels (12), which suggests that it may play a role in cardiovascular aging. Aging is an emergent property of complex systems, and it is fascinating to see how alterations in large molecules can affect aging-like outcomes.

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ENVIRONMENTAL SCIENCE

Water in the Balance

James S. Famiglietti^{1,2,3} and Matthew Rodell⁴

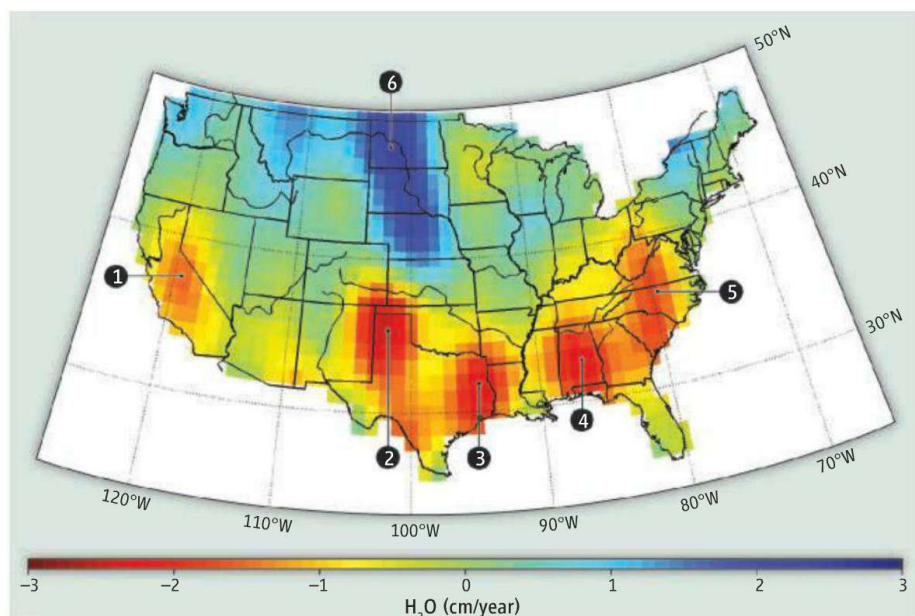
Satellite data may enable improved management of regional groundwater reserves.

Earth's climate is changing, and so is its hydrologic cycle. Recent decades have witnessed rising rates of global precipitation, evaporation, and freshwater discharge (1). Extreme flooding is occurring with greater intensity and frequency in some regions; in others, extreme drought is becoming more common (2). Most climate models indicate that by the end of this century, the dry regions of the world will become drier, whereas the wet areas will become wetter (3). Meanwhile, groundwater reserves, the traditional backup for water supplies during

extended periods of drought, are in decline globally (4–6). GRACE (the Gravity Recovery and Climate Experiment, a joint U.S.-German satellite mission) monitors these variations on monthly to decadal time scales, providing detailed data on the water cycle that are an essential prerequisite for contemporary water management.

Since its launch in 2002, GRACE has mapped monthly changes in Earth's gravity field with unprecedented accuracy (7). The main process driving the measured gravitational variations at monthly time scales is the redistribution of water, allowing GRACE to monitor changes in freshwater resources on land. For regions of 200,000 km² or more, GRACE functions as a giant "scale in the sky" weighing the total amount of water (snow, surface water, groundwater, and soil moisture) that enters or leaves a region each month with

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Mixed picture. Between 2003 and 2012, GRACE data show water losses in agricultural regions such as California's Central Valley (1) (-1.5 ± 0.1 cm/year) and the Southern High Plains Aquifer (2) (-2.5 ± 0.2 cm/year), caused by overreliance on groundwater to supply irrigation water. Regions where groundwater is being depleted as a result of prolonged drought include Houston (3) (-2.3 ± 0.6 cm/year), Alabama (4) (-2.1 ± 0.8 cm/year), and the Mid-Atlantic states (5) (-1.8 ± 0.6 cm/year). Water storage is increasing in the flood-prone Upper Missouri River basin (6) (2.5 ± 0.2 cm/year). See fig. S1 for monthly time series for all hot spots. Data from (15) and from GRACE data release CSR RL05.

an accuracy of 1.5 cm equivalent water height.

Because GRACE measures changes in total water storage, it integrates the impacts of natural climate fluctuations, global change, and human water use, including groundwater extraction, which in many parts of the world is unmeasured and unmanaged. GRACE-derived rates of groundwater losses in the world's major aquifer systems (4–6) underscore the critical need to improve monitoring and regulation of groundwater systems before they run dry.

Regional flooding and drought are driven by the surplus or deficit of water in a river basin or an aquifer, yet few hydrologic observing networks yield sufficient data for comprehensive monitoring of changes in the total amount of water stored in a region. GRACE observations have helped to fill this gap. They have been used to characterize regional flood potential (8) and to assess water storage deficits in the U.S. Drought Monitor (9) and are included in annual State of the Climate reports (10). As an integrated measure of all surface and groundwater storage changes, GRACE data implicitly contain a record of seasonal to interannual water storage variations that can likely be exploited to lengthen early warning periods for regional flood and drought prediction (see the figure).

The lack of comprehensive measurements also makes large-scale hydrological models,

key tools for predicting future water availability, difficult to validate. Low-resolution GRACE data, when combined with higher-resolution model simulations, provide an independent constraint on simulated water balances, while also adding spatial detail to GRACE's low-resolution perspective (11). They are widely used to evaluate land surface models used by weather and climate forecasting centers around the world (12).

Evapotranspiration is a key factor in interbasin water allocations, yet because it disperses into the atmosphere in the vapor phase, it confounds standard measurement techniques. The ability of GRACE to weigh changes in water stored in an entire river basin allows evapotranspiration to be estimated in a water balance framework (13).

Transboundary water availability issues require sharing hydrologic data across political boundaries. However, national hydrological records are often withheld for political, socioeconomic, and defense purposes, complicating regional water management discussions. Several studies have used GRACE data to circumvent international data denial practices, including in those involving lakes (14), river basins (6), and aquifers (4, 6). Likewise, regional and global maps of emerging trends in water availability (see the figure) can underpin discussions of geopolitical water security, conflict, and water diplomacy (6).

Although it still collects 10 months of data per year, GRACE has long outlived its planned 5-year life span. The GRACE Follow-On (GRACE-FO) mission, planned for launch in 2017, should enable continued collection of critical water and related climate observations for at least a decade, forestalling potential data gaps before a more advanced satellite gravimetry system is developed and launched, as tentatively planned for the 2020s.

For GRACE and its successors to maximize their value for water management, key issues must be addressed. First, the current 2- to 6-month latency before GRACE data are released must be substantially reduced to enable their use in seasonal prediction. Second, GRACE data should be better integrated into the modeling and decision support systems used by operational water management centers. Finally, next-generation missions beyond GRACE-FO should aim to achieve higher spatial ($<50,000$ km²) and temporal (weekly or biweekly) resolution, for example through novel orbital configurations, so that smaller river basins and aquifers can be observed directly. The availability of GRACE data at these finer scales, at which most planning decisions are made, would likely ensure their broader use in water management.

The GRACE-FO mission is on schedule for a 2017 launch, but a next-generation, improved GRACE mission is still under design and as yet unconfirmed. Given its demonstrated contributions to date and the potential for much more, a future without a GRACE mission in orbit would be an unfortunate and unnecessarily risky backward step for regional water management.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1236460/DC1
Fig. S1

10.1126/science.1236460

Cerebral Asymmetry and Language Development: Cause, Correlate, or Consequence?

Dorothy V. M. Bishop*

Background: Most children learn language effortlessly, but a minority struggle to master their native tongue for no obvious reason. This is known as specific language impairment. Affected children often have trouble learning to read and may also be diagnosed with developmental dyslexia. These language and literacy impairments are highly heritable, but their neurobiological basis is poorly understood. A popular notion is that the usual pattern of left-sided brain lateralization for language is disrupted in some children, which leads to problems with language and literacy. Until recently, it was difficult to test this idea, because we lacked easy methods to assess cerebral asymmetry directly in children.

Advances: Functional transcranial Doppler ultrasound has been developed as a method for assessing relative blood flow to the left and right cerebral hemispheres during a language-activation task. This technique has made it much easier to assess cerebral asymmetry directly and has confirmed the reduction of the usual left-sided language lateralization in specific language impairment and dyslexia. Studies performed with functional magnetic resonance imaging have provided converging evidence. The challenge now is to understand the nature of this association.



Functional transcranial Doppler ultrasound allows imaging of blood flow to the left and right sides of the brain. Still image from a video. [CREDIT: *Journal of Visualized Experiments*; see Additional Resources]

Nevertheless, we need to question common scientific views that ignore evidence for a substantial effect of nongenetic influences on individual differences in brain lateralization. Furthermore, cerebral lateralization is not fixed from birth; it can change with age. Thus, it seems likely that atypical cerebral asymmetry in language and literacy impairments is at least partly a consequence of poor language development.

Outlook: We now have the tools available to examine lateralization of language processing in real time. Yet, we still do not know whether cerebral asymmetry is a unitary trait or a multidimensional construct, nor how it changes as language develops. Before we can grasp the opportunities presented by technological developments in neuroscience and genetics, we need basic research to clarify how best to conceptualize and reliably measure cerebral asymmetry. Only then will we be able to reconcile the paradoxical findings that those with language and literacy impairments have atypical cerebral lateralization, yet in the general population, most individuals with atypical cerebral lateralization have typical cognitive development.

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READ THE FULL ARTICLE ONLINE
<http://dx.doi.org/10.1126/science.1230531>

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ARTICLE OUTLINE

Are Language and Literacy Impairments Associated with Atypical Cerebral Lateralization?

Do Certain Genes Influence Cerebral Lateralization?

Is Atypical Cerebral Lateralization Associated with Cognitive Deficits?

Is Atypical Cerebral Asymmetry a Contributory Factor for Language and Literacy Impairments?

Phenotypic Variation: Is Cerebral Lateralization a Unitary Variable?

Does Cerebral Lateralization Change with Age and Language Development?

Overview: Alternatives to the Endophenotype Account

Future Directions

ADDITIONAL RESOURCES

K. Hugdahl, R. Westerhausen, Eds., *The Two Halves of the Brain: Information Processing in the Cerebral Hemispheres* (MIT Press, Cambridge, MA, 2010).

YouTube channel dedicated to raising awareness of language-learning impairments. This channel includes short videos explaining what specific language impairment is (including international versions in 10 languages), how it is diagnosed, and how it relates to dyslexia:
www.youtube.com/RALLIcampaign

Visualized journal article demonstrating how functional transcranial Doppler ultrasound is used to assess cerebral lateralization in children (the figure is from this video):
www.jove.com/video/2161/assessment-cerebral-lateralization-children-using-functional

Web site giving background to functional transcranial Doppler ultrasound, including a simulator developed by Rune Aaslid, which allows you to see the kinds of signals recorded with this method. This site focuses on medical applications, rather than use for assessing cerebral lateralization:
www.transcranial.com/

Neurodevelopment
 Part of an occasional series
www.sciencemag.org/extra/neurodev

Cerebral Asymmetry and Language Development: Cause, Correlate, or Consequence?

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In most people, language is processed predominantly by the left hemisphere of the brain, but we do not know how or why. A popular view is that developmental language disorders result from a poorly lateralized brain, but until recently, evidence has been weak and indirect. Modern neuroimaging methods have made it possible to study normal and abnormal development of lateralized function in the developing brain and have confirmed links with language and literacy impairments. However, there is little evidence that weak cerebral lateralization has common genetic origins with language and literacy impairments. Our understanding of the association between atypical language lateralization and developmental disorders may benefit if we reconceptualize the nature of cerebral asymmetry to recognize its multidimensionality and consider variation in lateralization over developmental time. Contrary to popular belief, cerebral lateralization may not be a highly heritable, stable characteristic of individuals; rather, weak lateralization may be a consequence of impaired language learning.

Human language is distinct from other animal communication systems in its complexity, yet it is acquired effortlessly by children. We have known since the 19th century that language depends on specialized brain systems in the left cerebral hemisphere (1), and we are starting to understand the neurological basis of language networks at macroscopic, microscopic, and molecular levels (2, 3).

The claim that children acquire language effortlessly needs qualification, however. For some, mastery of their native tongue is a struggle: These children may be slow to produce their first words and, subsequently, may never achieve the grammatical complexity and fluent understanding shown by their peers. Although such difficulties can occur as the consequence of a genetic syndrome, neurological disease, or hearing loss, usually they have no obvious cause (4). This kind of unexplained, selective problem with language learning is known as specific language impairment (SLI).

There are close links between SLI and developmental dyslexia. Most children with oral language impairments have difficulty learning to read, and it is not uncommon for children with SLI to be identified as dyslexic as they attain school age (5). It would be misleading to suggest that SLI and dyslexia are the same thing, but there is considerable overlap between the two conditions, and very often the specific diagnostic label that is given is more a function of the person making the diagnosis than the actual profile of impairment. For this reason, the term “language and literacy

impairment” will be used here to refer generically to both conditions.

The neurobiological basis of both dyslexia and SLI remains unclear. For understandable reasons, brain-imaging studies with affected children are rare. Studies that have been done seldom find any gross evidence of structural brain abnormality, though differences from controls have been reported for proportions of gray matter in specific regions and for gyral morphology (6–8). However, these associations are probabilistic: There is substantial heterogeneity among affected individuals, and abnormalities that are found are seldom specific to language and literacy impairment.

One recurring theme in research on the neurobiology of language and literacy impairments is the idea that disruption of the normal pattern of left-hemisphere language lateralization may be implicated. This makes intuitive sense: A lateralized brain appears to have evolved in humans under strong selection pressure, yet left-hemisphere language is not universal in all people. It follows that, if a lateralized brain facilitates language learning, then a failure of lateralization could be a cause of poor language development.

Are Language and Literacy Impairments Associated with Atypical Cerebral Lateralization?

As early as 1925, Orton (9) had suggested that developmental reading problems might be the result of a poorly lateralized brain. This link was developed more fully in the 1980s, when Annett formulated a detailed genetic theory of lateralization that linked developmental dyslexia and language disorders to specific genotypes of a postulated “right shift” factor that was thought to influence both speech laterality and handedness (10). The field was constrained, however, by difficulties in direct assessment of cerebral lateral-

ization for speech. Before the development of modern neuroimaging, the only reliable way of assessing cerebral lateralization in an individual was the Wada technique, an invasive presurgical procedure in which anesthetic is injected into one carotid artery to produce a transient inactivation of the corresponding hemisphere (11). This was clearly inappropriate for research use with children, and for many years, most studies of laterality in children with developmental disorders focused on handedness as a proxy measure. However, this measure is far from ideal, as it is an indirect and imprecise indicator of lateralization for speech and language, which is not clearly associated with language and literacy problems (Box 1).

With the advent of neuroimaging, it became possible to look more directly at the brain in children. Yet, most studies considered only structural brain asymmetries. Claims for reduced asymmetry of the planum temporale (a region important for receptive language) in dyslexia produced initial excitement, but later studies gave a more confusing picture, and results appeared to depend on precisely how asymmetry is measured (12) and also on the type of specific learning disability (13). Furthermore, planum temporale asymmetry is not correlated with the lateralization of the brain response to a language-activation task, unlike some other asymmetries of brain structure (14, 15).

It became easier to study cerebral lateralization when functional transcranial Doppler ultrasound (fTCD) was adapted for imaging blood flow in the left and right middle cerebral arteries simultaneously while people performed language tasks (16). A word-generation task (for example, “think of as many words as possible beginning with the letter B”) typically induces enhanced blood flow to the left hemisphere for a few seconds after the task is initiated. This method is adequately reliable, and results correspond well with those obtained with the Wada technique (17).

When adults with SLI (18) and university students with developmental dyslexia (19) performed the word-generation task, both groups displayed reduced language laterality. Weak lateralization was not explained by poor task performance in either study. Subsequently, a handful of studies used functional magnetic resonance imaging (fMRI) to measure language lateralization in children or adults with language and literacy impairments, mostly finding that cerebral lateralization was weaker than in typically developing individuals (20–22).

Do Certain Genes Influence Cerebral Lateralization?

Developmental language and literacy disorders are heritable, and the pattern of inheritance sug-

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gests they are complex multifactorial disorders, caused by the combined action of many genes and environmental risk factors, each of small effect (23). Findings vary according to how the phenotype is defined: For children about whom there is some clinical concern, twin studies estimate heritability of language impairment at ~70%. Developmental dyslexia yields similar estimates (24), and there is some overlap in genetic variants that confer risk for language and literacy problems (25). This raises the possibility that the genetic risk for dyslexia and SLI could be mediated via an effect on cerebral lateralization, as shown in the endophenotype model of Fig. 1. The pleiotropy model (Fig. 1) shows an alternative genetic explanation, which postulates that the same genes that act as risk factors for language and literacy problems also affect cerebral lateralization. However, this explanation does not entail that cerebral lateralization mediates the relationship between genes and language and literacy problems. One difference between the two theories concerns the strength of the predicted relationship between genes and cerebral asymmetry. If cerebral asymmetry is a mediating factor, then we would expect to see stronger links between genes and cerebral asymmetry than between genes and language and literacy problems (26). This is not the case for the pleiotropy account.

Specific genes are known to affect asymmetric development in various animal species, notably worms, fish, and songbirds (27); however, our interest here is not so much in overall population biases as in individual differences in cerebral lateralization. Certain genes may lead to lateral biases of the body form in humans, but these genes may not display allelic variation; observed individual differences could be determined by environmental factors or chance. Thus, the key question is whether or not humans have polymorphic genes (i.e., genes that take different forms in different people), where the specific version of the gene affects cerebral asymmetry. One way of quantifying the role of genes in accounting for individual variation in brain asymmetry is to compare phenotypic resemblance in people with different degrees of genetic similarity. Twins provide a useful natural experiment because one can compare monozygotic (MZ) twins, who are genetically identical, with dizygotic (DZ) twins, who share, on average, 50% of alleles from polymorphic genes. Insofar as genes are important in determining a phenotype, one should see greater phenotypic similarity between MZ twin pairs than DZ twin pairs. In one study, Geschwind *et al.* measured frontal, temporal, parietal, and occipital brain volumes of 72 MZ and 67 DZ twin pairs (28). Estimates of heritability were high for raw brain volumes, but no figures were reported for heritability of brain asymmetries. Other studies of MZ twins have examined laterality indices for brain regions that show reliable structural asymmetries and have found only weak correlations between genetically identical individuals (29, 30).

In the largest brain-imaging twin study to date, Jahanshad *et al.* used diffusion tensor imaging (DTI) to measure structural integrity of white matter fibers in the left and right sides of the brain (31). Heritability was computed with maximum likelihood methods (32) for an asymmetry

index based on brain images from 11 regions taken from 60 MZ twin pairs and 45 same-sex DZ pairs. Just two regions gave heritability estimates greater than 0.25, and only one of these (the anterior thalamic radiation) had a superior fit for a model that included a genetic term, with a heritability

Box 1. Handedness, cerebral lateralization, and language and literacy impairment: myth and reality.

It is commonly believed that left-handedness is associated with conditions such as dyslexia and that handedness is highly heritable, and yet the evidence for both claims is lacking. In a comprehensive review of the literature, I found no association between handedness and language and literacy impairments (88). Twin studies confirm that there is some genetic influence on handedness, but ~75% of the variance is nongenetic and specific to the individual, with only 25% explained by genes (33). Molecular genetics studies have found significant associations with handedness, but the effects are complex and small in magnitude, suggesting a complex polygenic etiology rather than a single gene that determines an individual's handedness (89). Furthermore, the relation between handedness and language laterality, though significant, is imperfect: ~96% of right-handers have left hemisphere speech, compared with ~70% of left-handers (90). For these reasons, research using handedness as a proxy for cerebral lateralization will not be included in this Review.

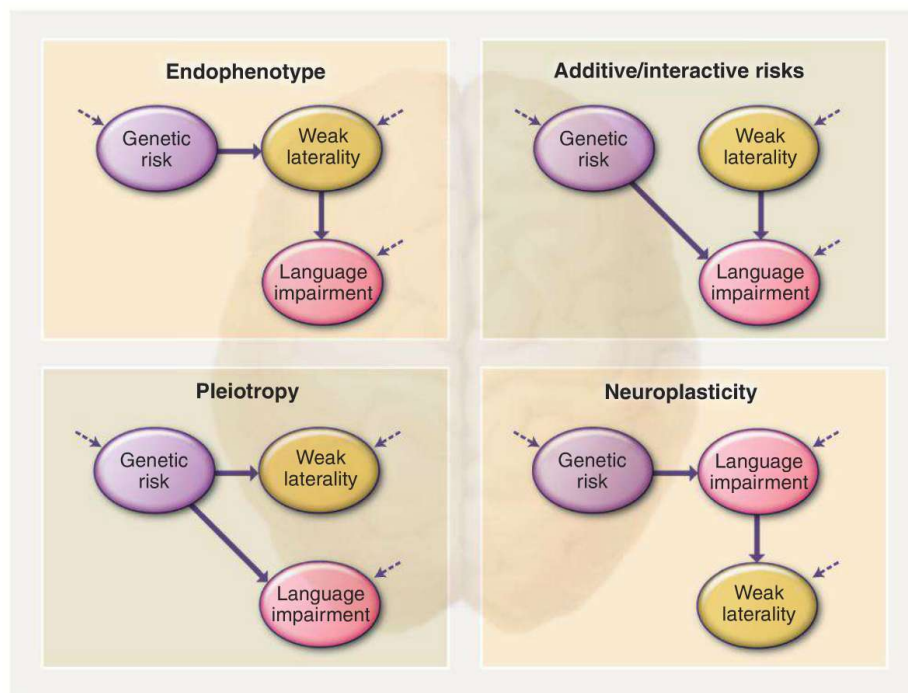


Fig. 1. Four path diagrams of the association between weak cerebral lateralization for language and language and literacy problems. These causal models are simplified abstractions and not mutually exclusive, but they illustrate the differing predictions about the pattern of associations that might be found between genotypes, language and literacy impairment, and cerebral lateralization. The endophenotype model depicts the situation in which genes that influence risk for language impairment do so by affecting cerebral lateralization. This model predicts that cerebral lateralization should be at least as heritable as language impairment, with the same genes affecting both traits. The pleiotropy model similarly assumes that the same genes that lead to risk for language impairment also affect cerebral lateralization, but there is no direct causal link: Weak laterality and language impairment co-occur because they have common origins, not because one causes the other. In the additive/interactive risks model, the genetic risk factors for language impairment do not affect lateralization. However, weak laterality, which could have genetic and/or nongenetic origins, exerts an independent causal influence on language impairment, which may add or interact with other genetic risk factors. In the neuroplasticity model, cerebral lateralization has no causal effect on language; rather, language impairment influences how the brain develops and is associated with weaker cerebral lateralization. The dotted arrows indicate that, for each construct, there will be sources of variation in addition to those depicted in the model.

estimate of 0.38. Interpretation of the study is complicated by low power to detect small genetic effects and by the fact that estimates of heritability may have been inflated because only right-handers were included. We know that brain asymmetry measured by DTI is discordant in ~29% of MZ twin pairs with opposite handedness, who constitute around one in five of all MZ pairs (33); in contrast, discordant brain asymmetry is seen in only 7% of those concordant for right-handedness (29). Note, however, that this study considered only structural brain asymmetry, and it is unclear how well this reflects functional language laterality. The few studies to look at associations between structural and functional asymmetry had small sample sizes (26 participants or less) and inconsistent findings (34–36).

Badzakova-Trajkov *et al.* considered functional brain asymmetry for MZ twins who were given three different tasks: a word generation task, lateralized to the left, and two nonverbal tasks that showed rightward asymmetry (37). The analysis focused on how concordance for language and visuospatial tasks varied in relation to concordance for handedness, but if one ignores handedness and collapses across groups, statistical analysis shows that these pairs of genetically identical twins were no more similar than is expected by

chance. Thus, evidence for heritability of functional brain asymmetry seems no stronger than that for structural asymmetries.

An alternative approach is to look directly for association between cerebral lateralization and specific genetic variants associated with language and literacy impairments. To evaluate such candidates, I searched the literature for neuroimaging studies that investigated structural or functional correlates of seven genes with common variants that had been associated with SLI and/or dyslexia (25). In addition, the *FOXP2* gene was included. Rare mutations in *FOXP2* are well established as a cause of severe speech and language disorder. More recently, common polymorphisms have been tentatively linked to language impairment (38).

When conducting an analysis of this kind, it is easy to neglect negative studies, because associations with asymmetry get mentioned only when significant. To minimize such bias, I searched Web of Science and Google to identify studies of brain correlates of polymorphisms in these candidate genes in healthy volunteers, regardless of whether or not asymmetry was explicitly mentioned in the title or abstract of each paper. I found nine studies focusing on the genes *CNTNAP2* (39–44), *DCDC2* (45), *DYX1C1* (45), *FOXP2* (46), *KIAA0319*

(45, 46), and *MRPL19/C2ORF3* (47). I did not find any studies for *ATP2C2* or *CMIP*.

The specific genotypes and phenotypes studied displayed wide variation. Because a single gene can have many functional polymorphisms, it is generally recommended to study haplotypes (i.e., patterns of alleles) (26), but this was seldom done. Some studies focused on just one or two single-nucleotide polymorphisms (SNPs) in a gene, whereas others evaluated several SNPs at different positions along the gene. Figure 2 summarizes the main findings. For *FOXP2*, 35 of the 37 SNPs tested by Pinel *et al.* (46) were not associated with brain activation. Of the other two, one (rs6980093) was associated with activation in the left frontal region, but those with different genotypes did not differ on an asymmetry measure. In the same study, 18 SNPs in a region on chromosome 6p22 spanning *KIAA0319*, *THEM2*, and *TTRAP* were studied in relation to brain activation during a reading task and a language-comprehension task. One SNP in *KIAA0319* was significantly associated with reduction of left-hemisphere asymmetry during reading. In a different study, Darki *et al.* found significant genetic variation in white matter volume in the left temporoparietal region for another SNP of *KIAA0319*, but the researchers did not explicitly test for brain asymmetry (45).

CNTNAP2 stands out in Fig. 2 as showing some evidence of lateralized effects in six of the eight samples that were tested for association with SNPs in this gene. Nevertheless, inconsistencies between studies cast doubt on the robustness of the results. Three studies considered SNP rs2710102, which has previously been associated with early language acquisition in autistic (48) and non-autistic samples (49) and also with a measure of nonword repetition in children with language impairments (50). In one study, 16 adults with the CC genotype showed reduced activation of the homolog of Broca's area in the right hemisphere when performing a sentence-completion task in the scanner (41). Scott-van Zeeland *et al.* studied association with risk and nonrisk genotypes in 32 children who performed a nonverbal implicit learning task. A left-frontal network was activated in the nonrisk group, but a more bilateral network was activated in the risk group (39). In a replication sample, 39 children were scanned while performing a different language-learning task, and two regions were identified as showing similar genetic effects. However, in contrast to other studies, this study grouped both CC and CT together as risk genotypes, with TT as nonrisk. A subsequent study failed to replicate these results, using the more usual definition of CC as risk genotype and CT/TT as nonrisk (42). Using a complex graph theory analysis of network connectivity, Dennis *et al.* found significant differences between risk and nonrisk genotypes, but, contrary to expectation, the CC genotype appeared to be associated with more efficient networks in both hemispheres.

The other neuroimaging studies of *CNTNAP2* focused on another SNP, rs7794745. An initial

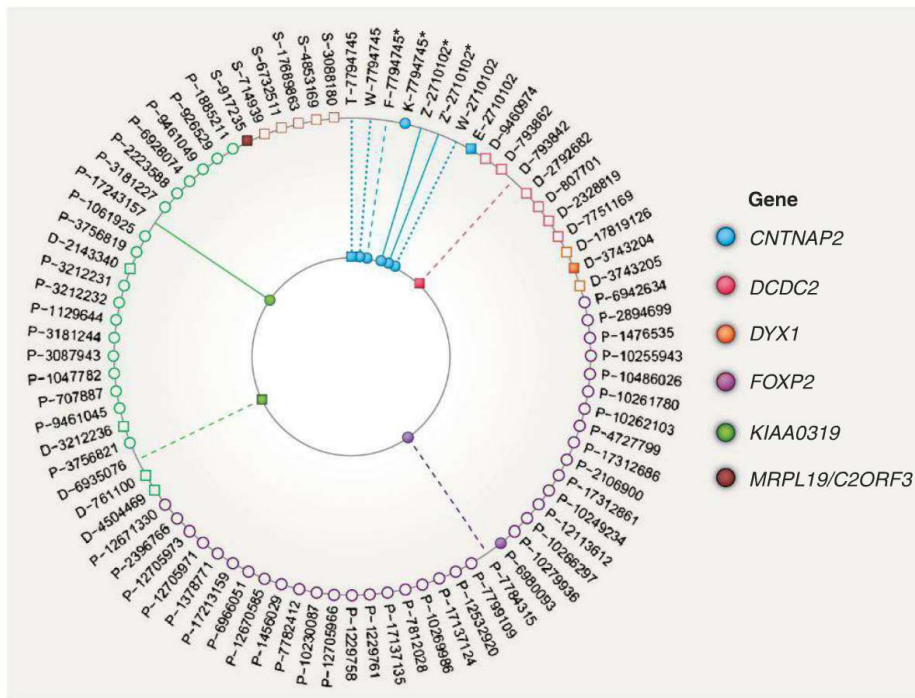


Fig. 2. Summary of effects of language- and/or reading-associated SNPs on asymmetry of brain structure or function. Unfilled shapes on the rim of the figure correspond to SNPs with no effect on brain structure (squares) or function (circles). Filled shapes on the rim correspond to a bilateral effect. For the remaining shapes, a solid line indicates reduced asymmetry for the risk genotype, a dashed line indicates reduction of left-hemisphere size or function, and a dotted line indicates other lateralized brain difference. Genes correspond to color-coding of the dots. SNP reference identification numbers are shown on the rim of the figure, with the study identified by letter. Asterisks denote nonstandard definitions of risk genotype (see text). The study codes are: T, Tan *et al.* (40); Z, Scott-Van Zeeland *et al.* (39) discovery sample; Z', Scott-van Zeeland *et al.* (39) replication sample; F, Folia *et al.* (43); W, Whalley *et al.* (41); E, Dennis *et al.* (42); D, Darki *et al.* (45); K, Kos *et al.* (44); P, Pinel *et al.* (46); S, Scerri *et al.* (same sample as D) (47). See text for more details.

report had associated this SNP with autism, but in a complex manner, with parent-of-origin effects (51). However, the association with autism failed to replicate (52), and this SNP has not been linked to language delay (49, 52) or SLI (50). A structural imaging study of a sample of 314 adults by Tan *et al.* obtained a complex pattern of results that included reduced gray and white matter on the right side in those with the TT versus AT/AA genotypes (40). In contrast, Whalley *et al.* (41) reported increased activation in the right middle temporal gyrus in those with the TT risk genotype ($n = 8$ people) compared with 57 people with AT/AA. Two other studies grouped TT and AT genotypes together into a risk group and compared them with the AA genotype. However, one study was too small to be meaningful (43), and the other found reduced electrophysiological responses to grammatical incongruity in those with TT/AT ($n = 23$) compared with AA genotypes ($n = 26$) but no evidence of lateralized differences.

Thus, whereas lateralized differences have been reported by different researchers linked with *CNTNAP2* variants, the categorization of genotypes is inconsistent from study to study, as are the results. Furthermore, given that the aim is to identify an endophenotype that might help explain why a genetic variant is associated with neurodevelopmental disorders, it is surprising that only two studies (41, 45) stated how genotype related to the cognitive abilities of their participants. In one of these studies, Darki *et al.* (45) found that white matter volume in left-hemisphere regions was significantly correlated with both genotype and reading ability. Furthermore, the differences in brain volume in relation to genotype were substantial (effect size = 0.8), suggesting that a laterality index based on these regions might be a candidate for further study as a potential endophenotype.

Overall, the evidence for genes affecting individual differences in cerebral lateralization is not strong, both in terms of twin study data and genetic variants associated with phenotypic variation. We cannot, however, dismiss the idea that genetic variants are implicated in cerebral lateralization. There are several problems to grapple with. First, most studies have focused on structural brain asymmetry rather than functional asymmetry. Second, where functional asymmetry is assessed, there is little consistency in how it is quantified. Third, established genetic associations between genes and language and literacy impairments are typically small in magnitude, with effect sizes for the difference between risk and nonrisk genotypes less than 0.2 (Cohen's d statistic). Very large sample sizes (more than 1000) are needed to detect effects of this magnitude; all of the neuroimaging studies reviewed here used much smaller samples and had the power to detect only large effects.

Given all of these limitations, it would be premature to conclude that there is no genetic influence on cerebral asymmetry, but the evidence to date is not promising for the endophenotype account (Fig. 1). The pleiotropy account could be tested using family data, insofar as the cross-trait

correlation between laterality and language or literacy impairment between individuals should depend on the strength of genetic relationship between them (53). However, for either model to be plausible, we would need better evidence that genes that affect language and literacy impairment also have reliable effects on cerebral asymmetry. Current evidence suggests that, just as with handedness (54), genetic variants may play a measurable, but relatively small, role in accounting for individual variation in lateralization.

Is Atypical Cerebral Lateralization Associated with Cognitive Deficits?

Perhaps the greatest challenge for any causal account comes from studies that look at the association between atypical cerebral lateralization and impairment in a different way: starting with individuals selected for atypical cerebral lateralization and assessing their language and literacy skills. In one such study, skills in a range of domains—including mastery of foreign languages, academic achievement, and verbal fluency—were not correlated with language lateralization in 31 people with right-hemisphere language, 31 with bilateral language, and 264 with left-hemisphere language (55).

Two smaller studies with children from the general population did, however, find some evidence of benefit of left-sided language lateralization. In a Canadian study, structural brain asymmetry (that is, laterality of the arcuate fasciculus measured on diffusion tensor imaging) was modestly correlated (correlation coefficient $r = 0.32$) with receptive vocabulary in 68 children, with the highest scores for those with strong left lateralization (56). Our group found similar results in a study that used fTCD to assess functional brain lateralization in 55 children, with a small but significant correlation of 0.34 between laterality index and vocabulary score (57). Nevertheless, in both studies there were several right-lateralized

children with mean vocabulary scores well above average. Additionally, a study of adults found no relation between left-lateralization of the arcuate fasciculus and vocabulary. On the contrary, in this study only 1 of 15 neuropsychological measures, a verbal memory task, showed any association with asymmetry of the arcuate fasciculus, and the effect was contrary to prediction, with worse performance associated with strong lateralization (58).

It would seem that atypical lateralization is compatible with normal or even above-average cognitive function, but in studies that oversample those with developmental difficulties, an association with language and literacy skills becomes apparent. There are two main lines of explanation that might account for this puzzling pattern of findings. First, atypical lateralization may add or interact with genetic risk for language and literacy impairments. Second, the asymmetry phenotype could differ for those who have language and literacy impairments and the remainder of the population.

Is Atypical Cerebral Asymmetry a Contributory Factor for Language and Literacy Impairments?

Figure 1 also illustrates the additive/interactive risks model, which treats atypical lateralization as a risk factor for language and literacy impairment while postulating that the origins of cerebral asymmetry are distinct from the genetic risk for language impairment. The idea here is that atypical cerebral asymmetry adds to or interacts with the genetic risk factors for language impairment. Figure 3 illustrates the logic. Let us consider a hypothetical genotype found in 30% of the population that, on average, depresses verbal skills so that for those with this risk genotype, the mean scaled score on a language measure is 94 rather than 100 (effect size, $d = 0.4$). In addition, a lack of left-sided cerebral lateralization (bilateral or right-hemisphere speech) is estimated

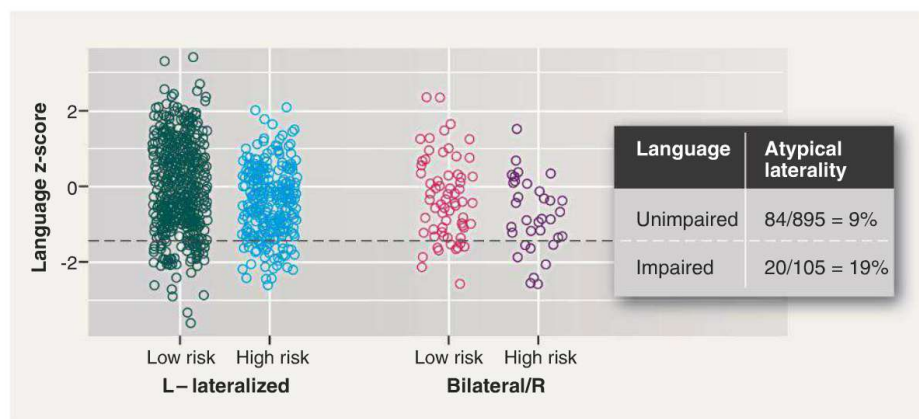


Fig. 3. Simulated data for a common genetic variant present in 30% of the population that depresses language z score by 0.4. In addition, language z scores are depressed by 0.4 in people with bilateral speech. The genetic variant and bilateral speech have independent origins, but their effects are additive. The rate of bilateral speech is 9% in those with unimpaired language (z score higher than -1.5) but is 19% in those with language impairment (z score less than or equal to -1.5). If the simulation is modified to give an interaction between genetic risk and bilateral speech, then the association between bilateral speech and language impairment becomes even stronger. L, left; R, right.

to affect 10% of people and has a similar impact. These effects are assumed to be independent and additive, so that the mean score for someone with both risks would be 88. Figure 3 shows data simulated with the use of these values. Overall, the rate of atypical cerebral asymmetry is roughly doubled among those with a language *z* score below -1.5 compared with the rest of the population, even though there is no significant correlation between laterality and language ability in the whole population. The impact of atypical cerebral asymmetry on language would be hard to detect in the general population because of the rarity of this phenotype. A power calculation shows that if there is a 9:1 ratio between two groups, a sample of ~ 600 cases would be needed to detect an effect size of 0.4 with 80% probability.

We can go further and suppose that the combination of atypical cerebral asymmetry and risk genotype might not be additive but instead interactive, such that the impact of the two factors together is more severe than would be predicted from each effect independently. This would further strengthen the association between atypical asymmetry and language impairment. However, the language deficit in those with atypical asymmetry would remain difficult to detect in samples from the general population, because those affected by the interactive effect would be in such a minority.

The important point here is that when an association is detected between traits in a clinical sample, it need not indicate shared origins. Genetic causes of language impairment and causes of cerebral lateralization could be quite independent: Co-occurrence of these phenotypes may be a consequence of how the sample was selected, if those with two risk factors are more likely to end up in the clinical group. According to this causal account, risk factors for atypical cerebral asymmetry, which could be nongenetic and/or genetic, are separate from genetic risks for language impairment.

Phenotypic Variation: Is Cerebral Lateralization a Unitary Variable?

Most people have visuospatial functions mediated by the right hemisphere and language by the left. This fits with the idea that cerebral lateralization allows for a division of labor between the hemispheres, with the left specializing in language, whereas the right specializes in visuospatial functions. In practice, however, some patients with focal brain lesions depart from this pattern, with both language and visuospatial functions controlled by the same hemisphere (59). Furthermore, the extent of left-hemisphere lateralization for verbal tasks is not strongly correlated with that of right-hemisphere lateralization for spatial processing, either in fMRI (60, 61) or fTCD studies (57, 62–64). It has been suggested that departures from complementarity simply reflect nonoptimal methods for computing a laterality index and consequent unreliability of laterality assessment (65): If measurement is noisy, then correlations between different indices may be swamped by

random error. However, some degree of dissociation between different indices of laterality appears to be the rule rather than the exception, even where asymmetry is measured with very little error. For instance, the measurement of handedness is highly reliable, yet handedness and structural brain asymmetries are often poorly correlated (66).

Indeed, studies that use multiple measures of cerebral lateralization suggest that, even within the domain of language, there can be dissociation between functions. Early studies with the Wada test found that some people showed opposite patterns of laterality for two language tasks: naming objects and saying a well-known series, such as days of the week (67). These cases were rare, and Wada testing is only performed with patients before epilepsy surgery, so one might dismiss this evidence as reflecting pathology rather than normal variation. Dissociation between different language functions can, however, be seen in the fTCD paradigm, where intercorrelations between laterality indices from different tasks are lower than the split-half reliability of each individual task (68). The evidence is not watertight: The key question is whether the correlation between the laterality index obtained with the same task given on different occasions (i.e., test-retest reliability) is higher than correlations that are observed between different tasks.

If we accept that cerebral lateralization is not a unitary function, this provides a possible resolution of the apparently contradictory findings, whereby individuals with language and literacy impairments have weak cerebral lateralization, but those with weak cerebral lateralization usually have normal cognitive skills. It may be that atypical lateralization can take different forms, only some of which are associated with poor functioning. For instance, could there be a cognitive disadvantage to having language and visuospatial functions mediated by the same hemisphere? To date, this notion has not received support, from either studies of neuropsychological patients (59) or fTCD data from 6- to 12-year-old children (57). It would, however, be interesting to test a related idea: that within the domain of language, there could be a disadvantage of having different linguistic functions distributed between the two hemispheres. Because it is typically assumed that language laterality is a unitary function, this idea has not been explored.

Does Cerebral Lateralization Change with Age and Language Development?

The final model in Fig. 1 is the neuroplasticity model, in which the causal path between cerebral lateralization and language and literacy development is reversed; that is, language ability influences cerebral lateralization. This may seem implausible, because structural brain asymmetries are evident in utero, long before the child learns language (69–73). However, this does not rule out a role of maturation or experience on functional laterality, given evidence from infants and toddlers that asymmetric processing of language

increases with age (74). A key question is whether this change simply reflects a maturational process under genetic control, or whether it is experience-dependent. Minagawa-Kawai *et al.* put forward a complex model that included a role for early perceptual asymmetries based on acoustic properties of auditory input, onto which was superimposed an increasing left-sided bias that developed as language engaged lateralized learning systems (74). Processing of phonemes in one's native language becomes increasingly left-lateralized, whereas detection of prosody becomes more right-lateralized with age. Linguistic status of sounds is critical in determining laterality: The same sound may be processed asymmetrically or symmetrically, depending on whether it is in the phonemic repertoire of the listener's native language. Pitch provides a particularly notable example: It is usually preferentially processed in the right hemisphere, but for learners of tone languages, where pitch is used phonemically to contrast meanings, a left-hemisphere processing bias emerges. Thus, developing cerebral asymmetry could reflect the growing engagement of lateralized systems for language learning and analysis as the child matures (74).

An earlier theory by Locke went further in linking a failure of cerebral lateralization to developmental language difficulties. He argued that if a child's language learning is disrupted or delayed for any reason, then left-hemisphere syntactic systems will fail to be engaged during a critical period of neurobiological development, and cerebral asymmetry will never be fully established (75).

Development of lateralization with age may involve topographic as well as directional changes (Fig. 4). Learning of perceptual and motor skills is sometimes associated with a more focal representation in the brain (76). Consistent with this, language-impaired children with poor phonological skills have more diffuse and bilateral processing of speech sounds than typically developing children (77). If focal representation develops as language competence improves, then we should see different patterns of bilateral cerebral representation, depending on language skill: In those with SLI, the bilaterality would reflect persistence of an immature pattern of diffuse activity, whereas in children with age-appropriate language skills, we should see activation of separate focal regions in left and right hemispheres (Fig. 4). To test this idea, we would need to move from treating cerebral lateralization as a single dimension to distinguishing different types of atypical lateralization, taking into account the spatial extent of activation associated with language functions in the two hemispheres.

Overview: Alternatives to the Endophenotype Account

There is growing support for the idea that atypical functional cerebral lateralization is associated with language and literacy impairments, but the work reviewed here challenges the conventional explanation for this association. At first glance, it

is tempting to think of cerebral lateralization as an endophenotype that mediates the relation between genetic risks and language and literacy impairments. However, there are difficulties for this account: First, most people with weak cerebral lateralization have no indications of cognitive deficits. Second, an endophenotype account would predict that risk genes should exert a stronger effect on cerebral asymmetry than on the language and literacy phenotype, yet the converse appears to be the case. The literature on genetics of cerebral lateralization is in its infancy, and we should not take lack of evidence for association as evidence of lack of association, especially as much of the evidence is from structural rather than functional brain asymmetry. Nevertheless, the data from twin studies suggest that, as with the related trait of handedness (54), individual differences in cerebral lateralization may turn out to be substantially influenced by nongenetic factors—possibly largely determined by random events occurring in early neurodevelopment (78). Genes almost certainly play a part in determining cerebral asymmetry, but their role is likely to be smaller and more probabilistic than generally assumed.

Alternatives to the endophenotype account are seldom considered, yet, as shown in Fig. 1, there are several other ways to account for the association between language and literacy impairments and cerebral asymmetry. Pleiotropic genes that exert separate influences on cerebral asymmetry and language development are one possibility, but here again, if heritability of cerebral asymmetry turns out to be much lower than that seen for language and literacy problems, it would be hard to fit existing data into such a model. An alternative is that cerebral asymmetry does not share genetic origins with language and literacy problems but acts as a separate risk factor that adds to or interacts with other risks. A final possibility is that atypical asymmetry in those with dyslexia or SLI is more a consequence than a cause of the language and literacy impairments. These possibilities are not mutually exclusive.

Future Directions

Measurement Issues

Even when structural brain asymmetries are the focus of research, there has been disagreement about how they should be measured and how far

they relate to functional asymmetries in handedness or language processing. Progress has been made, but systematic asymmetries can still be difficult to localize and distinguish from random fluctuations (66). This problem is magnified for functional measures of language lateralization. Furthermore, there is no agreement as to whether functional brain asymmetry is unidimensional or multifactorial, because when two laterality indices disagree, we are typically unable to tell if the difference is meaningful, a consequence of poor reliability of measurement (65), or indicative of genuine plasticity in functional lateralization. Now that methods such as fMRI and fTCD make it possible to study brain activity in healthy volunteers, we are in a position to do larger-scale studies that assess laterality for different tasks on repeated occasions, which will help us determine whether it is reasonable to derive a single scale from different measures or whether a spectrum of laterality measures is more appropriate.

The phenomenon of bilateral language processing is underresearched yet is of considerable interest because of claims of links to various neurodevelopmental disorders. If, as argued above, lateralization of different language functions can fractionate, then there may be different cognitive consequences for those who have opposite biases for different components of language, compared with those whose language processing is predominantly in one hemisphere. In addition, it may be important to distinguish between diffuse versus focal activation associated with language processing.

Genetic and Nongenetic Influences on Laterality

Once we have methods of adequate validity and reliability, we will be able to forge ahead with studies designed to identify genes that bias the human brain to left-sided language processing. The methods noted above, including twin and family studies, as well as molecular genetics, have potential to inform our understanding of the etiology of individual differences in lateralization. Genes known to be involved in determining the left-right axis of the body may be more promising candidates for this purpose than those implicated in language and literacy impairments (79).

It is worth pausing, however, to consider whether in the search for genes involved in cerebral lateralization we may have downplayed a role for nongenetic factors. These could potentially be of two kinds: systematic or stochastic. One systematic influence is the position of the fetus in utero, where, most commonly, the right ear faces outward (80). Potentially, a range of nongenetic prenatal influences could lead to small perceptual or motor asymmetries, which could, in turn, influence asymmetry of higher cognitive functions. The second kind of factor to consider is stochastic influences; that is, random fluctuations in the expression of individual genes (78). Even if we find genes that bias the human brain to left-sided language, we should not assume that variability in human asymmetry must be heritable: there could

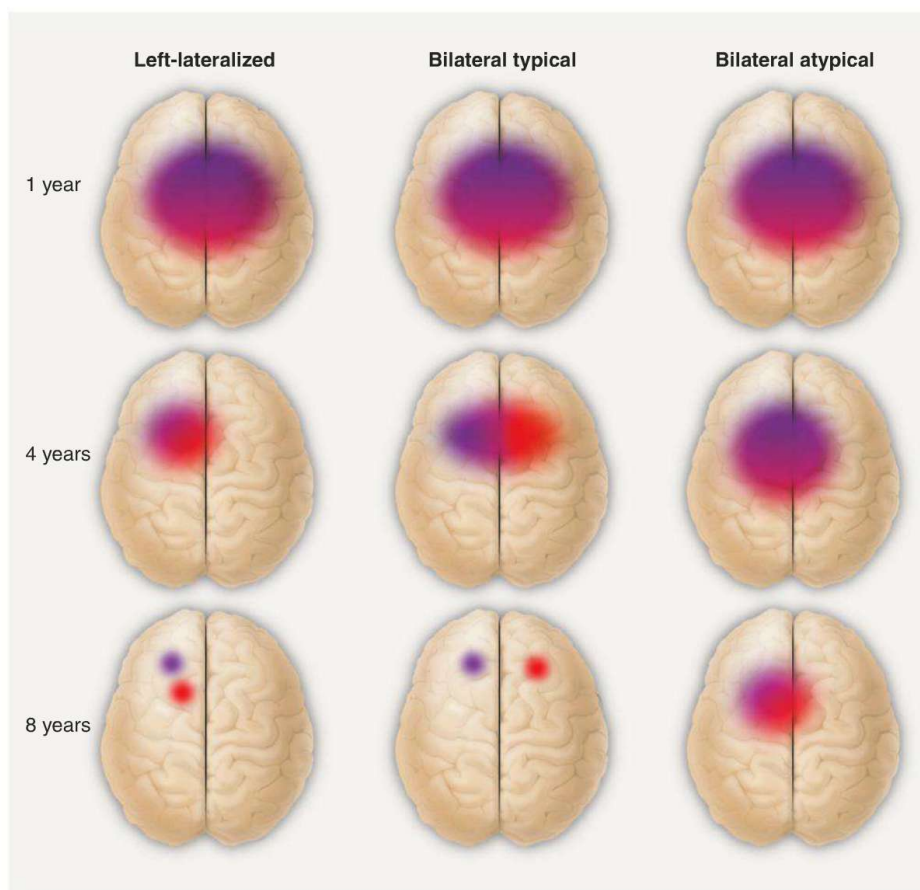


Fig. 4. Illustration of hypothetical distinction between different kinds of typical and atypical language lateralization. Red and purple regions correspond to different language functions (e.g., naming and series repetition, as examined in the Wada test). The typical developmental progression is from more diffuse language representation in infancy that steadily becomes more focal as skill develops and synaptic pruning occurs. Usually both language functions will be represented in the left hemisphere, but normal language function is compatible with separation into left and right hemispheres. For children with language impairments, the developmental progression to focal representation is much slower, if it occurs at all.

be a fixed gene (i.e., with no genetic variation) that has a probabilistic effect. Genetically identical individuals could show different degrees of asymmetry due to purely stochastic influences.

Studies of MZ twins could illuminate our understanding of cerebral asymmetry in two important ways. First, they can help identify environmental factors associated with phenotypic differences; for example, we could test whether prenatal positioning can account for postnatal differences in asymmetry. Second, these studies may help us distinguish between the causal models outlined here. For instance, can we find MZ twins who are concordant for language and literacy impairments, yet discordant for cerebral asymmetry? A consideration of the associations between traits both within and between related individuals can be helpful in distinguishing causal models (53).

Although language is specific to humans, lateralization of vocal behavior is not. Songbirds, notably zebra finches, provide an intriguing model organism for studying biological bases of structural and functional brain asymmetry (81). This work has potential for our understanding of the impact of genes on cerebral lateralization: The genome of the zebra finch has been mapped, and much is known about genetic influences on song learning (82), as well as the role of experience (87). It has even been suggested that there may be overlap in the mechanisms involved in song-learning in birds and language-learning in humans (83). A natural next step would be to test how gene expression relates to lateralization in the zebra finch brain.

Neuroplasticity of Cerebral Lateralization

Work with songbirds has emphasized that cerebral asymmetry is not fixed at birth, but can change substantially throughout development and be subject to experience. We still understand relatively little about such processes in humans, and we are limited by ethical considerations that preclude the kinds of experiential manipulations that are used with other species. Nevertheless, we can learn a great deal from the study of natural experiments. There is already fascinating research with deaf children learning sign language, indicating that the processing of a visual language shows similar lateralization to the processing of a spoken language (84). But what of children who have milder hearing impairments, who learn oral language but suffer from language delays (85), or those who have cochlear implants, where language outcomes are highly variable (86)? Are the language delays typically associated with hearing impairment accompanied by a delay in establishing language laterality? Another group of interest is children who can hear normally but cannot speak because of physical or motor impairment affecting the articulators. We know that, where there is adequate general intelligence, it is possible for a child with cerebral palsy to develop good grammatical and phonological skills, even in the case of total anarthria (inability to speak) (87). It would be fascinating to study cerebral lateralization in

such children while they perform a phonological judgment task, to establish whether their good receptive language skills are underpinned by an asymmetric language system. Finally, with development of child-friendly methods such as fTCD, we can begin to look at development of lateralization in late-talkers (that is, children who have few spoken words around 2 years of age, many of whom catch up with their peer group by the age of 4 or 5 years) to consider how cerebral asymmetry changes as language skills are acquired.

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Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge

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Introduction: Evolution of extended breath-hold endurance enables the exploitation of the aquatic niche by numerous mammalian lineages and is accomplished by elevated body oxygen stores and morphological and physiological adaptations that promote their economical use. High muscle myoglobin concentrations in particular are mechanistically linked with an extended dive capacity phenotype, yet little is known regarding the molecular and biochemical underpinnings of this key specialization. We modeled the evolutionary history of this respiratory pigment over 200 million years of mammalian evolution to elucidate the development of maximal diving capacity during the major mammalian land-to-water transitions.

Methods: We first determined the relationship between maximum myoglobin concentration and its sequence-derived net surface charge across living mammalian taxa. By using ancestral sequence reconstruction, we then traced myoglobin net surface charge across a 130-species phylogeny to infer ancestral myoglobin muscle concentrations. Last, we estimated maximum dive time in extinct transitional species on the basis of the relationship of this variable with muscle myoglobin concentration and body mass in extant diving mammals.

Results: We reveal an adaptive molecular signature of elevated myoglobin net surface charge in all lineages of mammalian divers with an extended aquatic history—from 16-g water shrews to 80,000-kg whales—that correlates with exponential increases in muscle myoglobin concentrations. Integration of this data with body mass predicts 82% of maximal dive-time variation across all degrees of diving ability in living mammals.

Discussion: We suggest that the convergent evolution of high myoglobin net surface charge in mammalian divers increases intermolecular electrostatic repulsion, permitting higher muscle oxygen storage capacities without potentially deleterious self-association of the protein. Together with fossil body-mass estimates, our evolutionary reconstruction permits detailed assessments of maximal submergence times and potential foraging ecologies of early transitional ancestors of cetaceans, pinnipeds, and sea cows. Our findings support amphibious ancestries for echidnas, talpid moles, hyraxes, and elephants, thereby not only establishing the earliest land-to-water transition among placental mammals but also providing a new perspective on the evolution of myoglobin, arguably the best-known protein.

FIGURES IN THE FULL ARTICLE

Fig. 1. Myoglobin net surface charge and maximal muscle concentration in terrestrial, semiaquatic, and aquatic mammals.

Fig. 2. Relationship between electrophoretic mobility and modeled myoglobin net surface charge.

Fig. 3. Inferring maximal myoglobin concentrations through myoglobin net surface charge across the mammalian phylogeny.

Fig. 4. Details of myoglobin net surface charge evolution in major groups of diving mammals.

Fig. 5. Evolution of myoglobin net surface charge and aquatic habits in Afrotheria.

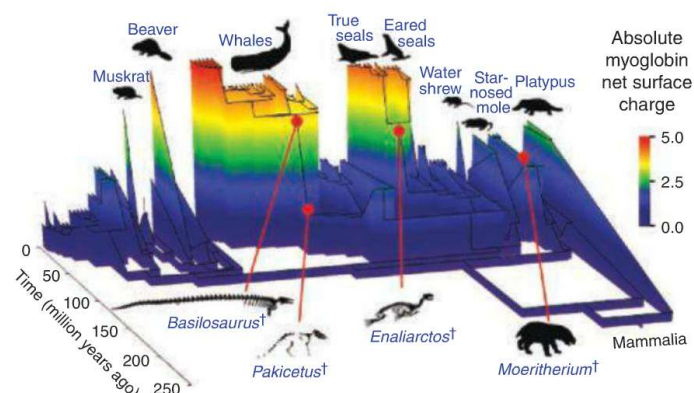
Fig. 6. Modeling diving capacity in ancestral whales, seals, and sea cows.

SUPPLEMENTARY MATERIALS

Supplementary Text
 Figs. S1 to S6
 Tables S1 to S7
 References

ADDITIONAL RESOURCES

video.sciencemag.org/VideoLab/diving/



Evolutionary reconstruction of myoglobin net surface charge in terrestrial and aquatic mammals. The figure reveals a molecular signature of elevated myoglobin net surface charge in all lineages of living elite mammalian divers with an extended aquatic history (upper silhouettes). This signature is used here to infer the diving capacity of extinct species representing stages during mammalian land-to-water transitions (†).

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Evolution of Mammalian Diving Capacity Traced by Myoglobin Net Surface Charge

Scott Mirceta,¹ Anthony V. Signore,² Jennifer M. Burns,³ Andrew R. Cossins,¹ Kevin L. Campbell,² Michael Berenbrink^{1*}

Extended breath-hold endurance enables the exploitation of the aquatic niche by numerous mammalian lineages and is accomplished by elevated body oxygen stores and adaptations that promote their economical use. However, little is known regarding the molecular and evolutionary underpinnings of the high muscle myoglobin concentration phenotype of divers. We used ancestral sequence reconstruction to trace the evolution of this oxygen-storing protein across a 130-species mammalian phylogeny and reveal an adaptive molecular signature of elevated myoglobin net surface charge in diving species that is mechanistically linked with maximal myoglobin concentration. This observation provides insights into the tempo and routes to enhanced dive capacity evolution within the ancestors of each major mammalian aquatic lineage and infers amphibious ancestries of echidnas, moles, hyraxes, and elephants, offering a fresh perspective on the evolution of this iconic respiratory pigment.

Expert mammalian divers, such as Northern elephant seals and sperm whales, are able to hold their breath and actively forage in the oceans for periods of well over an hour (1, 2). Even for less-proficient aquatic or amphibious mammals, maximal dive duration defines maximal foraging depth, enables the exploitation of new food sources, determines how far polar marine or temperate freshwater species can venture under ice cover, and limits how long they can stay submerged for predator evasion (3–5).

The fossil record has revealed highly convergent changes in body, limb, and tail structures accompanying the secondary transitions of mammals to an aquatic habitat (6). Comparative physiological analyses of extant divers have further illustrated that the attendant reductions in the energetic costs of underwater locomotion are coupled with greatly elevated body oxygen stores and their economical use (4, 7, 8). At the onset of submergence, an enhanced dive response leads to cessation of breathing, reduced heart rate, and peripheral vasoconstriction. The latter largely isolates nonessential organs from the central blood oxygen supply, which is spared for the oxygen-sensitive brain and heart and exercising muscles. Most dives stay aerobic in nature, but during dives approaching maximal capacity accumulation of carbon dioxide and lactic acid may lead to severe acidosis (9). Maximal active dive time ($t_{\max}$) has been linked to the maximal skeletal muscle concentration of myoglobin, $[Mb]_{\max}$, which

varies greatly among divers for poorly understood reasons (4, 10, 11).

Myoglobin (Mb) of the sperm whale was the first protein characterized at the atomic level in Nobel prize-winning work (12) and is perhaps the best studied of all proteins. Mammalian Mbs are small, 153–amino acid globular proteins whose primary to tertiary structures are highly conserved, giving rise to eight alpha helices that enclose a hydrophobic core containing the oxygen-binding heme group and simultaneously provide a highly polar external surface. Mb is involved in the storage and facilitated diffusion of oxygen within muscle cells (13). It has a higher oxygen affinity than hemoglobin and is little affected by pH and other allosteric effectors. Although these biochemical properties are conserved across terrestrial and aquatic mammals (14), Mb and its precursor, apo-Mb (without the heme), are more stable against unfolding and aggregate less easily at high con-

centrations in aquatic relative to terrestrial mammals, consistent with much higher expression yields of sperm whale Mb in *Escherichia coli* compared with Mbs of terrestrial mammals (15, 16).

We show that high $[Mb]_{\max}$ values across all mammals are highly correlated with increased Mb net surface charge (Z_{Mb}) obtained by modeling Mb primary structure onto the tertiary structure of the protein and using site-specific, conserved ionization constants. By using ancestral sequence reconstruction, we traced the evolution of Z_{Mb} and hence $[Mb]_{\max}$ across the mammalian phylogeny. Combining this with allometric analysis of $t_{\max}$ and fossil body mass estimates, we reconstructed the evolution of diving capacity across the major groups of mammalian divers.

Results

Mb Net Surface Charge and Maximal Muscle Concentration

$[Mb]_{\max}$ of elite divers may exceed values in terrestrial mammals by factors of more than 30, reaching 100 mg/g of wet mass (Fig. 1A) or 133 mg/ml of water [assuming 75% (weight/weight) muscle water content]. High $[Mb]_{\max}$ in divers is invariably linked with an increase in Z_{Mb} , at pH = 6.5 from around +1 in terrestrial mammals to about +5 in elite divers (Fig. 1A). Although intracellular muscle pH in diving mammals is unknown, a similar pattern holds for pH = 6.0 and 7.0 and thus likely holds over the range of physiological pH values (fig. S1) (17). Our estimates of Z_{Mb} closely agree with the electrophoretic mobilities of purified Mbs from selected aquatic and terrestrial mammals (Fig. 2, A and B), confirming the reliability of modeling Z_{Mb} from Mb amino acid sequence and conserved tertiary structure and site-specific ionization constants. Protein solubility is generally lowest around zero net surface charge, increasing toward both higher and lower net surface charges (18). The positive Z_{Mb} in elite divers may cause electrostatic repulsion and thereby help to reduce the tendency of Mb and/or apo-Mb to

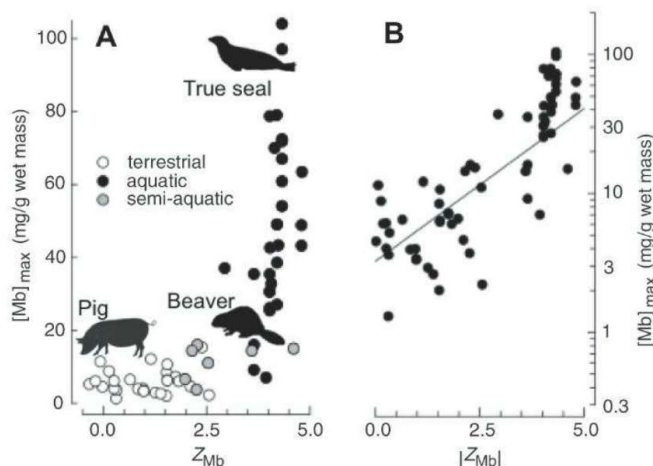


Fig. 1. Myoglobin net surface charge and maximal muscle concentration in terrestrial, semiaquatic, and aquatic mammals. (A) $[Mb]_{\max}$ as a function of Z_{Mb} and **(B)** semilogarithmic plot of $[Mb]_{\max}$ against $|Z_{Mb}|$; see text for the regression line (Eq. 1). Z_{Mb} was obtained by modeling at pH = 6.5. Silhouettes show representative terrestrial, semiaquatic, and aquatic mammals.

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self-associate (16, 19–21), which may otherwise interfere with its function in facilitated oxygen diffusion at high Mb concentrations in a heavily protein-crowded sarcoplasm (13, 21, 22).

After accounting for potential phylogenetic effects (23), the exponential increase in $[Mb]_{\max}$ with absolute Mb net charge, $|Z_{Mb}|$, is described by

$$\log[Mb]_{\max} = 0.220|Z_{Mb}| + 0.511 \quad (1)$$

[$P < 0.001$, regression under an Ornstein-Uhlenbeck process (Fig. 1B)]. This highly significant relationship allows estimates of $[Mb]_{\max}$ in species for which this information is unavailable (gaps in Fig. 3A) from their sequence-derived Z_{Mb} values (Fig. 3B and table S1). Moreover, with use of ancestral sequence reconstruction, Z_{Mb} and hence $[Mb]_{\max}$ can be estimated for extinct species along the mammalian phylogeny, allowing insights into the evolution of mammalian muscle oxygen storage capacities over 200 million years of their evolutionary history (Fig. 3C and fig. S2).

Highly Convergent Increase of Mb Net Surface Charge in Diving Mammals

Strong, recurrent increases in Z_{Mb} arose by charge-increasing substitutions at multiple sites and correspond with the adoption of both semi- and fully aquatic lifestyles. For example, the early evolution of cetaceans shows an increase of Z_{Mb} from +1.1 at 54 million years ago (Ma) to +3.7 at the time of the last common ancestor of baleen and toothed whales [36 Ma (Fig. 4A)]. This change in charge predicts a 3.5-fold increase in $[Mb]_{\max}$ from 6 mg/g in early Eocene *Pakicetus*-like cetaceans to 21 mg/g in late Eocene *Basilosaurus*-like modern whale ancestors (Eq. 1). Our analysis suggests that the increase in Z_{Mb} was restricted to the cetacean lineage and did not occur in a common ancestor of whales and their closest living relatives, the semiaquatic hippopotamuses (Fig. 4A), implying a low degree of aquatic specialization in their last common ancestor.

Notably convergent increases in Z_{Mb} were also observed in the Phocidae (true seals) and Otarioidea (eared seals and walrus) after initial increases in common pinniped ancestors of the group (Fig. 4B). This finding indicates secondary, independent increases in physiological diving capacity in both lineages after their divergence, concordant with fundamental differences in their mode of aquatic locomotion, body insulation, and foraging behaviors (24). Our analysis accordingly suggests a maximal Z_{Mb} value of +3.0 for the late Oligocene/early Miocene stem pinniped *Enaliarctos melesi* (25), corresponding to an $[Mb]_{\max}$ of 15 mg/g (Fig. 4B). This value is less than half of the value (38 mg/g) predicted by maximum parsimony-based reconstruction of $[Mb]_{\max}$ (fig. S3). Maximum parsimony fails to resolve the convergent nature of $[Mb]_{\max}$ increases during pinniped evolution, which illustrates the additional insights gained by our approach using the molecular signature of Z_{Mb} .

Otaroids, phocids, and cetaceans evolved steep increases in Z_{Mb} , which plateaued at values between about +4 and +5, a likely functional optimum (Fig. 4, A and B). This is supported by the convergent evolution of similarly high Z_{Mb} values in both beaver and muskrat (Fig. 4C). Other lineages of semiaquatic mammals, such as the diminutive American water shrew and star-nosed mole, show somewhat smaller increases in Z_{Mb} , or they do not differ substantially from their non-diving relatives (e.g., American mink and polar bear), matching their lower muscle Mb content (Figs. 1A and 3A) and more recent aquatic evolutionary history (Fig. 4, B to D).

Elevated $[Mb]_{\max}$ values in diving mammals are invariably accompanied by positive Z_{Mb} values, even though highly soluble proteins, such as serum albumin, are often characterized by strongly negative Z_{Mb} values (26). However, the existence of already slightly positive Z_{Mb} in most terrestrial mammals (Fig. 1A) may have selected against the accumulation of negatively charged substitutions upon evolving a diving lifestyle, because this would initially decrease absolute net surface charge and hence likely also Mb solubility.

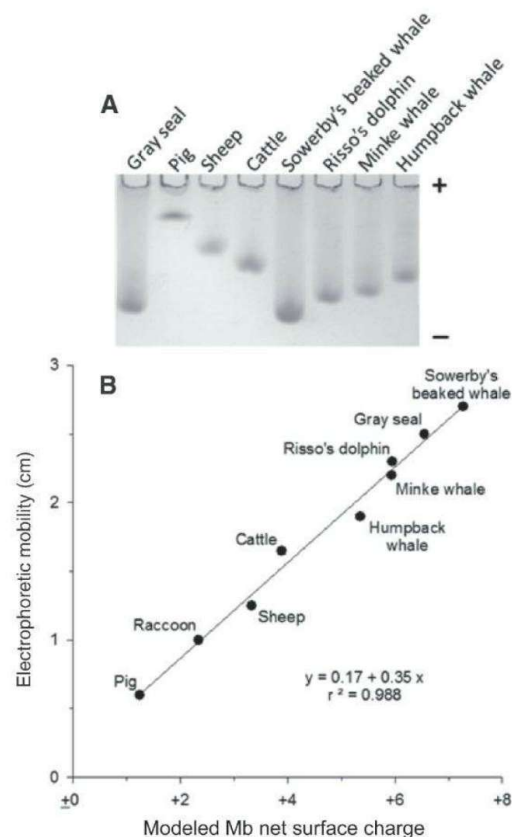
The observed charge-increasing substitutions frequently involve replacements of neutral amino acids with fully positively charged Lys (K) or weakly positively charged His (H) residues, for example, cetaceans, pinnipeds, beaver, and muskrat (Fig. 4, A to D). Both of these are regarded as essential dietary amino acids for mammals (27), and this may underlie the delayed increase in

muscle Mb concentration during development of diving mammals (11, 28), the submaximal Mb concentration in nonlocomotory muscles (28), and the relatively low $[Mb]_{\max}$ values in baleen whales (table S1), which may be under relaxed selection pressure for elevated muscle oxygen stores because of lower mass-specific metabolic rates at their gigantic size.

External surface His residues in Mb tend to have acid dissociation constants that result in only partial occupation of the proton binding site at physiological pH (table S2) and are therefore less efficient in increasing Z_{Mb} relative to gains of fully positively charged Lys (K) and Arg (R) [or losses of fully negatively charged Asp (D) and Glu (E)]. His residues will, however, increase the specific buffer value of the protein (29, 30) and thereby whole-muscle buffer power at the high Mb concentrations found in diving mammals. They will thus contribute to limiting pH changes, both during aerobic breath-hold dives, where carbon dioxide still accumulates, and at the end of long dives, where profound lactic-acidosis is observed (9). This is in line with the largest number of additional His residues (four) having evolved in the Mbs of elite diving beaked whales and phocid seals (Fig. 4, A and B).

Charge-increasing substitutions may occur at each of the external Mb surface positions (e.g., Fig. 4 and fig. S4). Of these, Asn (N) and Asp (D) at positions 12 and 122, respectively, are especially noteworthy, because they form a key stabilizing dimer contact in the recently resolved x-ray crys-

Fig. 2. Relationship between electrophoretic mobility and modeled myoglobin net surface charge. Native polyacrylamide gel of selected, purified mammalian Mbs at pH = 6.0 (A) and correlation between electrophoretic mobility and modeled Mb net surface charge (B).



tallographic structure of dimeric horse Mb (20) and are replaced particularly frequently [five and three times independently, respectively (Fig. 4)]. Their recurrent substitution in diving mammals may help reduce the tendency for dimer formation at high concentrations, as is indeed observed for sperm whale Mb (16). High Mb concentrations and Mb dimer formation have been shown to lower its translational diffusion coefficient (13, 19, 22), which may interfere with Mb's role in facilitated diffusion of oxygen inside muscle cells.

Recent Amphibious Ancestries of Extant Terrestrial Mammals?

The convergent increase of Z_{Mb} across living mammalian divers, including semiaquatic lineages such as water shrews and star-nosed moles, corroborates the predictive power of this molecular signature for inferring (semi)aquatic lifestyles. Thus, marked elevations in Z_{Mb} argue that an amphibious stage, still manifested in platypus and star-nosed mole lineages, immediately predated evolution of modern terrestrial monotremes and fossorial moles, respectively (Fig. 4, D and E). This inference is in line with previous independent suggestions of recent amphibious ancestries of echidnas (31) and talpid moles (32) using morphological data sets. Modern echidnas and moles are accomplished burrowers, which, like high-altitude mammals, are generally characterized by moderately elevated muscle Mb levels (28, 33). It is unlikely, however, that the strong ancestral increases in Z_{Mb} and therefore $[Mb]_{max}$ in these

two lineages were associated with hypoxic subterranean or high-altitude environments, because Z_{Mb} is low or only moderately elevated in extant mammals with extended burrowing or high-altitude evolutionary histories [e.g., burrowing naked and blind mole rats, aardvarks, and golden moles and high-altitude black lipped pikas, guinea pigs, yaks, and Tibetan antelopes (Fig. 4 and table S1)].

An amphibious ancestry of Proboscidea has also been debated for more than a century on the basis of fossil anatomy, depositional environment, and isotope data (34–37). However, it is still unclear whether this has evolved independently from or in a common ancestor with sirenians (sea cows) (38). We have therefore determined Mb primary structures from all extant sirenian and proboscidean genera, together with the extinct woolly mammoth and Steller's sea cow. Our reconstruction of Z_{Mb} shows three consecutive charge-increasing substitutions already in the late Cretaceous/early Paleocene last common ancestor of sea cows, elephants, and hyraxes [paenungulates (Fig. 5)]. Increases of Z_{Mb} of such magnitude are otherwise only observed in lineages of expert divers [cetaceans, pinnipeds, beaver, and muskrat (Fig. 3)].

An amphibious ancestry of hyraxes may appear surprising; however, past hyracoid diversity was much richer than indicated by the three small-bodied (<5 kg), strictly terrestrial genera of today, as illustrated by the at least two times larger *Pliohyrax kruppii* and the 390-kg *Kvabebihyrax kacheticus* from the Pliocene (2 to 5 Ma), for

which semiaquatic lifestyles have been proposed as long as a century ago (39, 40). Recent isotope evidence has further indicated a possible semiaquatic lifestyle for the late Eocene (37 Ma) *Thyrohyrax meyeri* and another, as yet unnamed, new hyracoid genus of similar age (34). The reconstruction of a high Z_{Mb} value in a hyracoid ancestor is not affected when ancestral sequence reconstruction is based on alternative phylogenetic hypotheses, such as hyraxes rather than sirenians comprising the sister group to proboscideans (41). This and recurrent gigantism [e.g., rhinoceros-sized *Titanohyrax ultimus* (42)] calls for a reassessment of the lifestyle of other fossil hyraxes that have previously been considered unambiguously terrestrial (37).

Paenungulates contain two other lineages of large, extinct mammals, the enigmatic Desmostylia and Embrithopoda, for which semiaquatic lifestyles have also been proposed (6, 43). Taken together, these results suggest a shared secondarily amphibious history of all paenungulates on the basis of the reconstructed molecular properties of their Mb, establishing this clade as the earliest placental mammal radiation into the aquatic niche more than 64 Ma.

Our analysis also indicates that muscle O_2 storage capacities in early amphibious proboscideans may have been even higher than in basal paenungulates, because two additional positive Mb charges are reconstructed in the stem proboscidean lineage, which are compensated by two negative charges in the last common ancestor of recent elephantids (Fig. 5). The most parsimonious evolutionary scenario in light of fossil evidence is that Z_{Mb} continued to increase in early proboscideans before a secondary reduction. Thus, late Eocene/early Oligocene semiaquatic *Moeritherium* (34) may have possessed a Z_{Mb} and muscle O_2 storage capacity comparable to those found in modern pinnipeds and cetaceans (Figs. 4 and 5). The observed charge-changing substitutions in the Cape hyrax lineage are similarly consistent with a Z_{Mb} peak that coincided with the emergence of the possibly semiaquatic *Thyrohyrax meyeri* (34) (Fig. 5).

Within sirenians, we reconstructed a secondary reduction in Z_{Mb} from the late Cretaceous/early Paleocene paenungulate ancestor toward modern forms, suggesting that semiaquatic early middle Eocene (50 Ma) sirenians, such as *Pezosiren portelli* (44), had higher muscle oxygen storage capacities than living forms, presumably because of the higher metabolic costs for functioning well both on land and in water in amphibious mammals (45). The very low Z_{Mb} of extant, fully aquatic manatees is fully in line with a negligible muscle oxygen storage capacity (46) and is seemingly linked to a strongly reduced metabolic rate (oxygen requirements) and a blunted dive response in these sluggish herbivores (46, 47). The secondary nature of these reductions is supported by observations on Steller's sea cow shortly before its extinction in 1768, where muscles “redder than the flesh of land animals” were noted and the

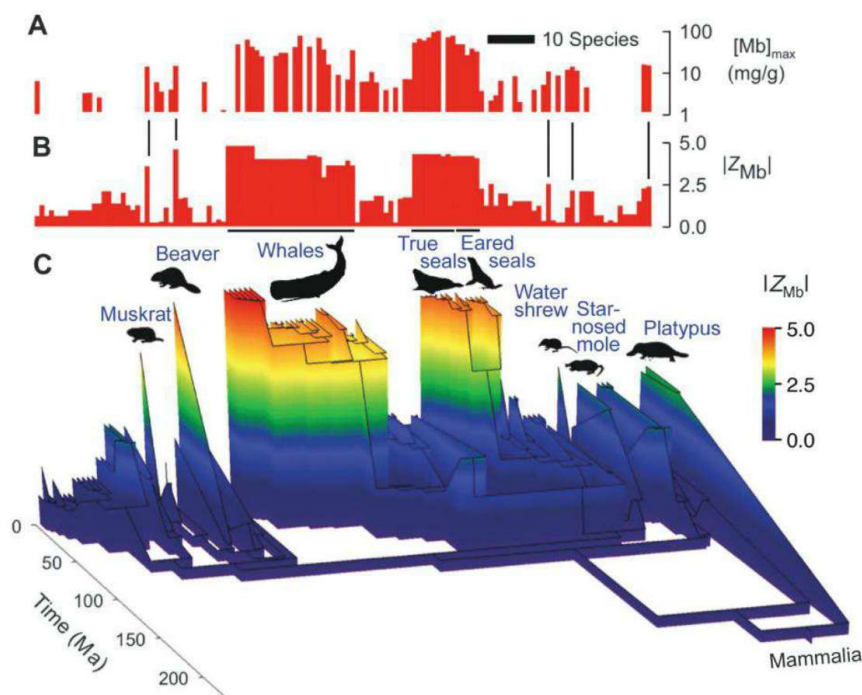


Fig. 3. Inferring maximal myoglobin concentrations through myoglobin net surface charge across the mammalian phylogeny. (A and B) The comparative availability of data on $[Mb]_{max}$ and $|Z_{Mb}|$, respectively, in extant mammals. (C) Three-dimensional evolutionary reconstruction of $|Z_{Mb}|$ (z axis, color-coded, at pH = 6.5) across their phylogeny (x-y plane). Silhouettes and names indicate recent taxa of mammalian divers with elevated Z_{Mb} values.

animals exhibited intermittent cessation of blood flow from their wounds upon facial submergence (48), a sign of peripheral vasoconstriction and a dive response.

Modeling Dive Capacity in Extinct Mammals

Our evolutionary reconstruction of Z_{Mb} allows estimation of $[Mb]_{max}$ and hence muscle oxygen storage capacities in extinct transitional forms representing mammalian land-to-water transitions. To gain insight into the likely attendant maximal diving capacities, we have plotted all available t_{max} values for extant mammals, covering the full complement of mammalian diving abilities (e.g., from moose to elephant seals), against body masses ranging from 16-g water shrews to 80,000-kg blue whales (Fig. 6). Size influences t_{max} , because

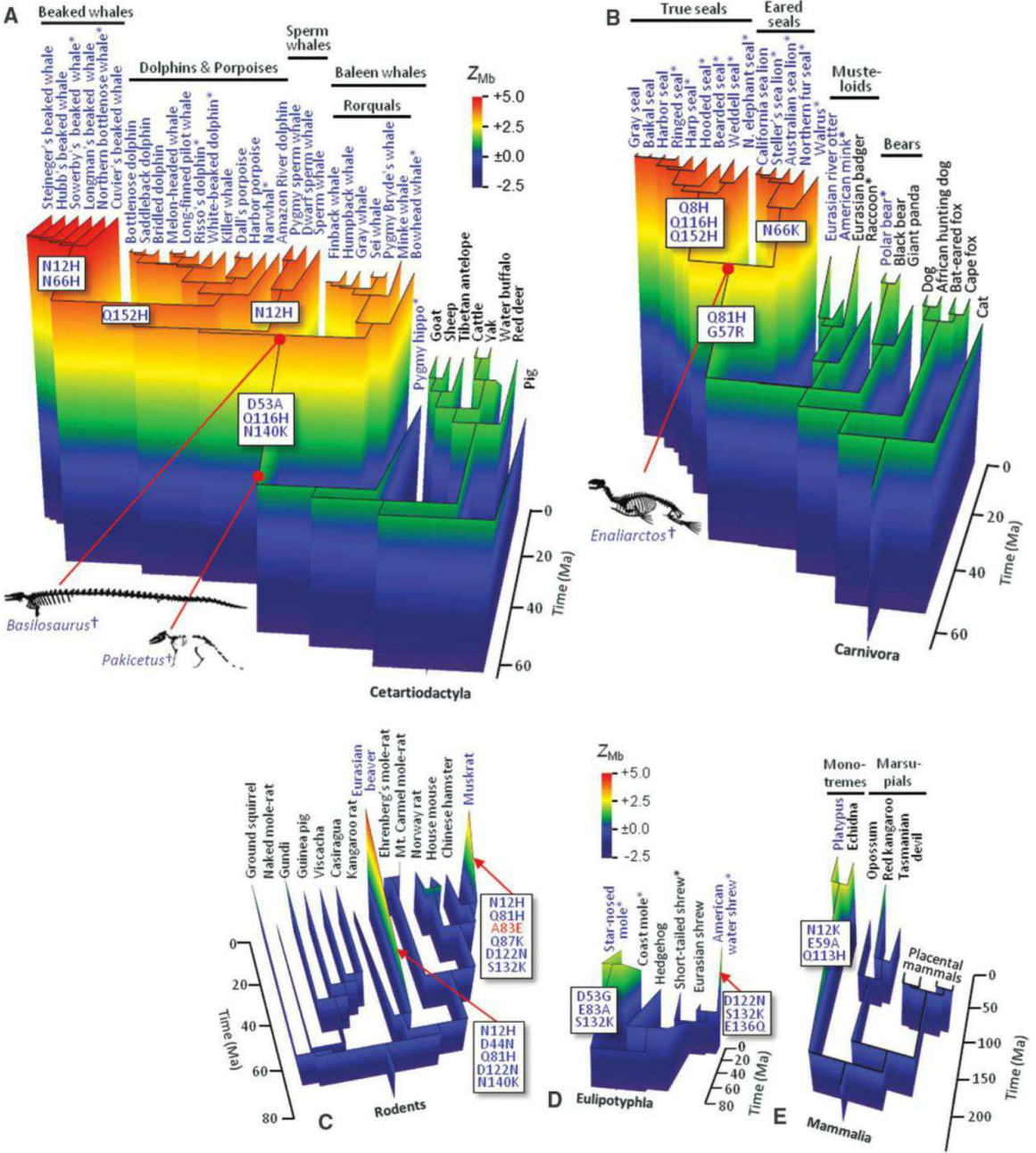
oxygen-storing organs in mammals (lung, blood, and muscle) generally increase isometrically with body mass, whereas metabolic rate increases with body mass exponents below 1.0, allowing larger mammals to deplete their oxygen stores more slowly (10, 49). Thus, the Eocene land-to-water transition of whales was accompanied by a progressive increase in body mass (Fig. 6A and table S3). However, Fig. 6 illustrates that, even at constant body mass, t_{max} varies greatly in living mammals. This variation can principally be ascribed to species-specific deviations from the allometric predictions of diving metabolic rate and/or available oxygen stores. The former is mostly unknown even for extant mammals, and the latter depends on the complex depletion dynamics of variably sized lung, blood, and muscle oxygen reservoirs

(4, 7, 8). Despite these difficulties, we find that a two-factor regression model with body mass (m , in kg) and $[Mb]_{max}$ (mg/g) as factors yields highly significant predictions of t_{max} (in s; ordinary least-squares regression $P < 0.001$ for intercept and slopes, $r^2 = 0.820$, with no significant interaction between factors $P = 0.793$):

$$\log t_{max} = 0.223 \log m + 0.972 \log [Mb]_{max} + 0.891$$
 (2)

The model predicts 82% of the observed variation in $\log t_{max}$ among the 42 extant mammals for which $[Mb]_{max}$, t_{max} , and body mass are available (table S1). The model is not significantly affected when potential phylogenetic effects are accounted for (23), indicating that the

Fig. 4. Details of myoglobin net surface charge evolution in major groups of diving mammals. (A) Cetartiodactyla, (B) Carnivora, (C) Rodentia, (D) Eulipotyphla, and (E) Monotremata and Marsupialia. Names of diving and terrestrial species are given in blue and black fonts, respectively. Selected key (>10.20) charge-increasing (blue font) and charge-decreasing (red font) amino acid substitutions are indicated. See fig. S2 for all charge-changing substitutions. Estimated Z_{Mb} values of selected extinct species (†) are also indicated. Asterisks mark Mb sequences determined in the present study. Skeletal reconstructions are modified from original publications (table S3).



relationship is fundamentally applicable across the mammalian phylogeny. Although variations in lung and blood oxygen stores and diving metabolic rate (4, 7, 8) are known to underlie differences in $t_{\max}$ among diving taxa, in the absence of such information we show that body mass and $[\text{Mb}]_{\max}$ together are good markers for the overall net effect of these factors on $t_{\max}$.

Combined with estimates of fossil body mass, our reconstruction of Z_{Mb} and hence $[\text{Mb}]_{\max}$ (Eq. 1) suggests an order of magnitude increase in $t_{\max}$ during the Eocene land-to-water transition of whales, from about 1.6 min in wolf-sized, early Eocene *Pakicetus* to about 17.4 min in the 6500-kg, late Eocene *Basilosaurus* (Fig. 6A and table S3). The latter $t_{\max}$ value notably falls within the range of extant oceanic dolphins and orquals but is still less than a third of the value in similar-sized modern beaked whales or the 10-fold larger sperm whale. Because deeper dives take longer and deep-foraging mammals generally show the longest maximum dive times (49, 50), this suggests that the rich mesopelagic food resources exploited by modern beaked and sperm whales may not yet have been fully available to late Eocene whale ancestors.

Reconstruction of diving capacity in the stem pinniped *Enaliarctos* yields a $t_{\max}$ value at the lower end of the range found in similar-sized modern eared seals that is comparable to the value in the threefold smaller Californian sea otter (Fig. 6B). This inference suggests *Enaliarctos* exploited shallow-water habitats as opposed to the deep foraging behavior of modern true seals. Within Sirenia, $t_{\max}$ likely changed little since the time of the semiaquatic quadruped *Pezosiren* (50 Ma) because the gradual reduction in Z_{Mb}

and associated $[\text{Mb}]_{\max}$ was accompanied by an increased body mass that culminated in the gigantic Steller's sea cow (Figs. 4 and 6C). Increased diving capacity presumably confers little selective advantage onto these shallow-feeding, slow-moving aquatic herbivores. In a rare case of a return from a secondarily semiaquatic to a terrestrial habit, diving capacity within Proboscidea decreased from an estimated 10 min in late Eocene/early Oligocene amphibious *Moeritherium* to about 2.5 min in terrestrial Asian elephants, consistent with a secondary reduction in Z_{Mb} and low $[\text{Mb}]_{\max}$ (Figs. 4 and 6C and tables S1 and S2).

Discussion

Our integrative, comparative evolutionary approach provides insights into a molecular signature of elevated Z_{Mb} that is found in all elite mammalian divers and correlates with exponential increases in $[\text{Mb}]_{\max}$ and the timing of physiological diving-capacity evolution across the major lineages of diving mammals. We show that a simple model based on body mass and maximum muscle Mb concentration provides very good estimates of maximum dive times in living, and therefore likely also in extinct, mammals across the whole range of diving abilities.

These results give insights into the potential foraging ecologies of early whale and seal ancestors and lead us to hypothesize recent amphibious phases in a number of living terrestrial mammalian lineages, which are open for future testing, for example, by isotope analysis of fossil tooth enamel and examination of fossil limb-bone densities (57).

Our results are further consistent with the view that the convergent evolution of high Z_{Mb} increases intermolecular electrostatic repulsion of

Mb and/or its less stable precursor, apo-Mb, permitting higher oxygen-storage capacities in the muscles of mammalian divers without potentially deleterious self-association of Mb or apo-Mb. Accordingly, aquatic mammals repeatedly evolved a natural form of protein "supercharging," the recombinant introduction of charged amino acid residues for increased protein solubility (52).

Recently, novel roles, such as in hypoxic vasodilation (53) and protection of the hypoxic brain (54), have been proposed for Mb. Integration of these and other physiological and biochemical traits with Mb sequence evolution across vertebrates may not only reveal mechanisms behind extreme physiological capacities but also improve our understanding of basic protein structure-function relationships.

Materials and Methods

Body Mass, Maximal Active Dive Time, and Maximal Muscle Mb Concentration

Mammalian body mass and $t_{\max}$ values were assembled from the literature, taking care to select maximum active dive times, i.e., foraging or traveling dives (table S1). $[\text{Mb}]_{\max}$ was determined spectrophotometrically (55) or taken from the literature (table S1). In some cases, $[\text{Mb}]_{\max}$ was determined by its correlation with muscle iron content obtained from the literature (table S4 and fig. S5).

Mb Net Surface Charge

Z_{Mb} was calculated as the sum of the charge of all ionizable groups (56) at pH = 6.5 by modeling Mb primary structures onto the tertiary structure and using published, conserved, site-specific ionization constants or ionization constants we determined from comparisons of acid-base titration curves of Mbs with known residue differences (table S2). In the latter case, Mb from selected species was purified from muscle tissues by using standard protein purification methods, deionized by gel filtration, and titrated as the cyanmetMb derivative under a CO₂-free atmosphere in 0.1 M KCl at 25°C by using 0.1 M NaOH and HCl between pH = 5 and 9. Unknown pK_a (where K_a is the acid dissociation constant) values were estimated from the difference in the titration curves obtained for Mbs with and without the ionizable group of interest. In this way, comparison with pig Mb yielded a pK_a value of 7.10 for His⁸⁸ in sheep. Similarly, comparison with dolphin Mb yielded a pK_a of 6.07 for His⁶⁶ in beaked whales. The purity of Mb preparations and the accuracy of modeled Z_{Mb} values were confirmed by assessing Mb electrophoretic mobility on native polyacrylamide gels for selected mammalian species, showing an excellent agreement between electrophoretic mobility and the modeled net surface charge of Mbs from terrestrial and aquatic mammals (Fig. 2, A and B).

Mb Sequences from Recent Mammals

These were obtained from DNA or complementary DNA (cDNA) after polymerase chain reaction

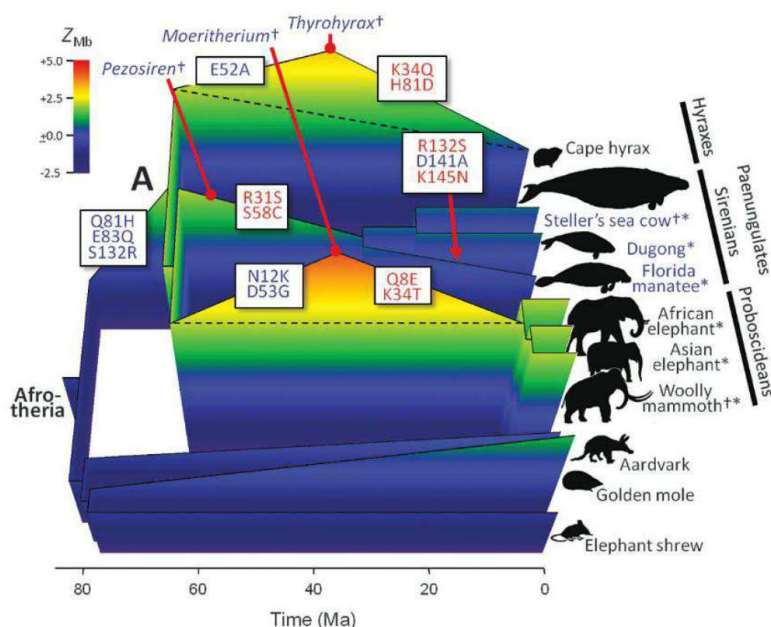


Fig. 5. Evolution of myoglobin net surface charge and aquatic habits in Afrotheria. Z_{Mb} values above dashed lines represent our preferred interpretation of the order in which the inferred charge-changing substitutions may have occurred. "A" denotes the inferred shared amphibious ancestor of paenungulates. Other explanations are as in Fig. 4.

(PCR) amplification. Ancient DNA from Steller's sea cow and woolly mammoth were obtained from 1000- to 1300- and ~28,000-year-old mandibles, respectively. See table S5 for sources of all tissue samples.

RNA Extraction for cDNA Synthesis

Total RNA was extracted from muscle homogenates by following the Trizol method [Invitrogen (Life Technologies Limited), Paisley, UK]. Concentration and quality of RNA was determined by using a NanoDrop ND-1000 spectrophotometer (Labtech, Uckfield, UK) and by 1.5% agarose gel electrophoresis. We then used 5 µg/ul of total RNA in a standard SuperScript II (Invitrogen) reverse transcriptase reaction to create first strand cDNA. For some species, it was necessary to use 5' and 3' rapid amplification of cDNA ends (RACE). For 5' RACE, the first-strand cDNA from the superscript II reaction was poly-C-tailed by using terminal transferase (New England Biolabs, Hitchin, UK), and excess primer and nucleotides were removed by using a MinElute PCR Purification Kit (Qiagen, Manchester, UK) according to the manufacturers' instructions. For 3' RACE, first-strand cDNA was synthesized by following the SMART method (Clontech, Oxford, UK).

PCR and Sequencing

Mb coding sequences were amplified from cDNA by PCR by using forward and reverse primers designed on the basis of consensus alignments in the 5' and 3' untranslated regions from closely related species (table S6). Eulipotyphlan Mb amplification was conducted by using primers to obtain a core fragment, followed by two 5' RACE PCR using a poly-G primer and gene-specific reverse primers STSR3 and STSR1 or CMR1, and 3' RACE, which was conducted by using the SMART primer and the consensus forward primer 3'. Mb probe, based on a conserved region between position 306 and 326 in mammalian Mb coding nucleotide sequences. Elephant Mb exons were amplified separately from genomic DNA (gDNA) by using conserved primers designed by Primer Premier software (version 5, Premier Biosoft, Palo Alto, CA) in the flanking regions (table S6). Partial woolly mammoth Mb sequences were assembled by using Sequencher software (version 4.6, Gene Codes, Ann Arbor, MI) from sequence fragments in the 0.8× nuclear genome database (<http://mammoth.psu.edu>) (57) mapped to the Asian elephant. Remaining gene-coding regions were amplified from gDNA in seven fragments (each 85 to 186 bp in length) by PCR in an ancient DNA dedicated laboratory (McMaster

University, Canada) using primers designed as above (table S6). Successful amplicons were purified, ligated into pDrive vectors, and transformed into EZ-Competent cells using a PCR Cloning^{plus} Kit (Qiagen). Cloned inserts were amplified by a 57°C colony PCR using primers M13-F and M13-R according to a standard PCR protocol (Invitrogen). All PCRs were carried out according to conditions described in table S7, and amplifications were confirmed on 1.5 to 2% agarose gels. Successful amplicons were purified as above and sequenced on an Applied Biosystems 3130 Genetic Analyzer using a BigDye Terminator v3.1 Cycle Sequencing Kit [Applied Biosystems (Life Technologies Limited), Warrington, UK]. Sequencing reads from elephants and the woolly mammoth were assembled by using Sequencher software (version 4.6), and the amino acid complement of the Mb gene was deduced from the consensus sequence.

Design of Sirenian Mb Hybridization Capture Array

An Agilent 244k SureSelect Capture Array (Agilent Technologies, Santa Clara, CA) was designed to capture the Mb genes from Florida manatee, dugong, and the extinct Steller's sea cow. Coding sequences of the Mb gene (plus 25 bp of up-

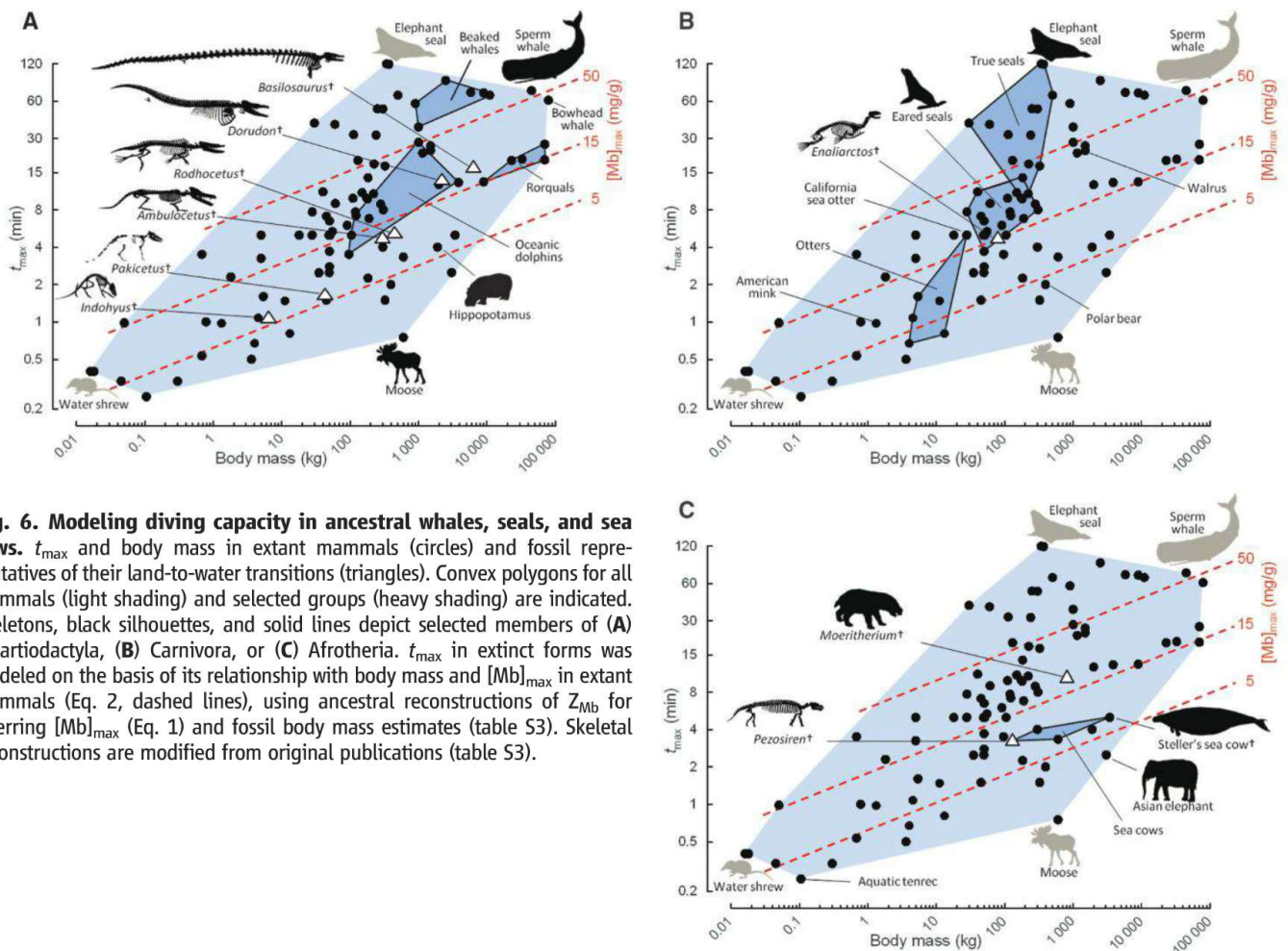


Fig. 6. Modeling diving capacity in ancestral whales, seals, and sea cows. $t_{\max}$ and body mass in extant mammals (circles) and fossil representatives of their land-to-water transitions (triangles). Convex polygons for all mammals (light shading) and selected groups (heavy shading) are indicated. Skeletons, black silhouettes, and solid lines depict selected members of (A) Cetartiodactyla, (B) Carnivora, or (C) Afrotheria. $t_{\max}$ in extinct forms was modeled on the basis of its relationship with body mass and $[Mb]_{\max}$ in extant mammals (Eq. 2, dashed lines), using ancestral reconstructions of Z_{Mb} for inferring $[Mb]_{\max}$ (Eq. 1) and fossil body mass estimates (table S3). Skeletal reconstructions are modified from original publications (table S3).

stream and downstream sequence of each of the three exons) mined from the genomic data sets of the African elephant and rock hyrax (GenBank accessions AAGU03077599.1, ABRQ01421420.1, ABRQ01421419.1, and ABRQ01816509.1) were used to design a series of overlapping 60-bp probes (1-bp tiling) by using Agilent's eArray tool (earray.chem.agilent.com). Probes were masked of repetitive elements by using RepeatMasker (www.repeatmasker.org) and imprinted on six identical microarrays by Agilent Technologies.

Construction of Indexed Sirenian DNA Library

Indexed DNA libraries suitable for Illumina (Illumina, San Diego, CA) sequencing were prepared from gDNA samples (table S5). A ~250-mg bone sample of Steller's sea cow specimen ZI6852 was ground to powder by using mortar and pestle in a dedicated ancient DNA laboratory at the University of York, and DNA was extracted by following the protocol described in Rohland *et al.* (58). Samples ZI6853 and ZI17170(2) were extracted from ground bone samples in an ancient DNA dedicated laboratory in Copenhagen by using the DNeasy Blood and Tissue Kit (Qiagen). Extraction blanks, serving as negative controls, were treated in a similar manner throughout. For the manatee and dugong samples, 200 ng of gDNA was fragmented by using NEBNext dsDNA Fragmentase (New England BioLabs) in 4.0- μ l reactions, following manufacturer's guidelines. Reactions were promptly purified with an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Pittsburgh, PA). Blunt-end repair, adaptor ligation, and adaptor fill-in reactions were performed on the fragmented DNA samples as previously described (59); reaction cleanup was by MinElute PCR purification kit (Qiagen). We added 10 μ l of each successful library preparation to indexing PCR reactions; for conditions, see table S7. PCR success was verified by 2.5% agarose gel electrophoresis and amplified products were purified with an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Life Sciences). Purified PCR products were split into four equal volumes and reamplified with primers IS5_reamp.P5 and IS6_reamp.P74, in reactions described for indexing PCR and purified with MinElute as above.

DNA libraries prepared from Steller's sea cow, dugong, and Florida manatee DNA were hybridized to custom designed capture arrays (60). Captured products were sequenced by Ambry Genetics (Aliso Viejo, CA) on an Illumina Genome Analyzer IIx using a 54-bp singleton sequencing protocol. Raw Illumina reads were trimmed of adapters and low-quality bases and then assembled to a reference sequence by using Geneious software (Biomatters Limited, Auckland, New Zealand). Because the draft genome of the Florida manatee has recently become publicly available, the complete Mb gene was mined from this data set (GenBank accession AHIN01036638.1) and used as the reference sequence for the

manatee, dugong, and Steller's sea cow Mb assemblies.

In cases where Mb primary structure deduced from DNA data differed from previously published amino acid sequences (e.g., Asian and African elephant, aardvark), the former was included in the analysis.

Ancestral Mb Sequences

These were reconstructed from the aligned Mb amino acid sequences of 128 extant and two recently extinct mammals (fig. S4) by the maximum likelihood method as implemented in MEGA5 (61) on a user-defined composite, time-calibrated mammalian phylogeny that had been assembled from literature data in Mesquite (62) (fig. S6; see supplementary text for sources and the tree in Newick format) (17). Ancestral sequence reconstructions used the Dayhoff+G model, which was determined as best fitting the data according to the model test facilities in MEGA5. Ancestral amino acid reconstructions were generally supported by probabilities of more than 90%, such that for ancestral charge reconstructions only the most probable amino acid for each site was considered. fig. S2 displays the modeled net charge at all ancestral nodes of the composite mammalian phylogeny and all inferred charge-changing Mb substitutions over the entire tree.

Modeling Maximal Active Dive Times and Statistics

We developed two regression models: first using Z_{Mb} as predictor of $[Mb]_{max}$ and second using body mass and $[Mb]_{max}$ together as predictors of t_{max} . For these analyses, $[Mb]_{max}$, body mass, and t_{max} were $\log_{10}$ -transformed. Because the hierarchical nature of phylogenetic relatedness in multispecies data sets potentially violates the assumption of statistical independence, we used likelihood ratio tests to determine whether ordinary least-squares regression, phylogenetic generalized least-squares regression, or regression under an Ornstein-Uhlenbeck process best fit our data with the program RegressionV2.m in MATLAB (MathWorks, Natick, MA) as previously outlined in detail (23). For this, branch lengths of the composite tree (fig. S6) were modified by the method of Nee in Mesquite (62) before analysis in RegressionV2.m. Statistical significance was accepted at $P < 0.05$.

t_{max} in ancestral mammals was modeled by first determining ancestral Z_{Mb} based on ancestral primary structure reconstruction (see above). Z_{Mb} was then used to obtain ancestral $[Mb]_{max}$, which together with literature data on fossil body mass (table S3) was then used to predict t_{max} , all based on the relationships here established between these parameters in extant mammals (Eqs. 1 and 2).

For comparison, we also used linear parsimony as implemented in MacClade 4.0 (63) to reconstruct ancestral $[Mb]_{max}$ as a continuous-valued character, as described before (29), with Carnivora as an example (fig. S3).

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Supplementary Materials

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Supplementary Text
Figs. S1 to S6
Tables S1 to S7
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Terahertz Metamaterials for Linear Polarization Conversion and Anomalous Refraction

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Polarization is one of the basic properties of electromagnetic waves conveying valuable information in signal transmission and sensitive measurements. Conventional methods for advanced polarization control impose demanding requirements on material properties and attain only limited performance. We demonstrated ultrathin, broadband, and highly efficient metamaterial-based terahertz polarization converters that are capable of rotating a linear polarization state into its orthogonal one. On the basis of these results, we created metamaterial structures capable of realizing near-perfect anomalous refraction. Our work opens new opportunities for creating high-performance photonic devices and enables emergent metamaterial functionalities for applications in the technologically difficult terahertz-frequency regime.

Control and manipulation of electromagnetic (EM) polarization states has greatly affected our daily life, from consumer

products to high-tech applications. Conventional state-of-the-art polarization converters use birefringence or total internal reflection effects (1) in

crystals and polymers, which cause phase retardation between the two orthogonally polarized wave components. Expanding their typically limited bandwidth requires complex designs using multilayered films or Fresnel rhombs. At microwave and millimeter-wave frequencies, narrow-band polarization converters have been constructed by using metallic structures, such as birefringent, multilayered meander-line gratings (2). Fabrication challenges and high losses render these unsuitable for optical frequencies (3).

Metamaterials have enabled the realization of many phenomena and functionalities unavailable through use of naturally occurring materials (4–6). Many basic metamaterial structures, such as metal split-ring resonators (7), exhibit birefringence suitable for polarization conversion (8–16), which has been mostly investigated in the microwave frequency range. Broadband metamaterial circular polarizers have been demonstrated in the

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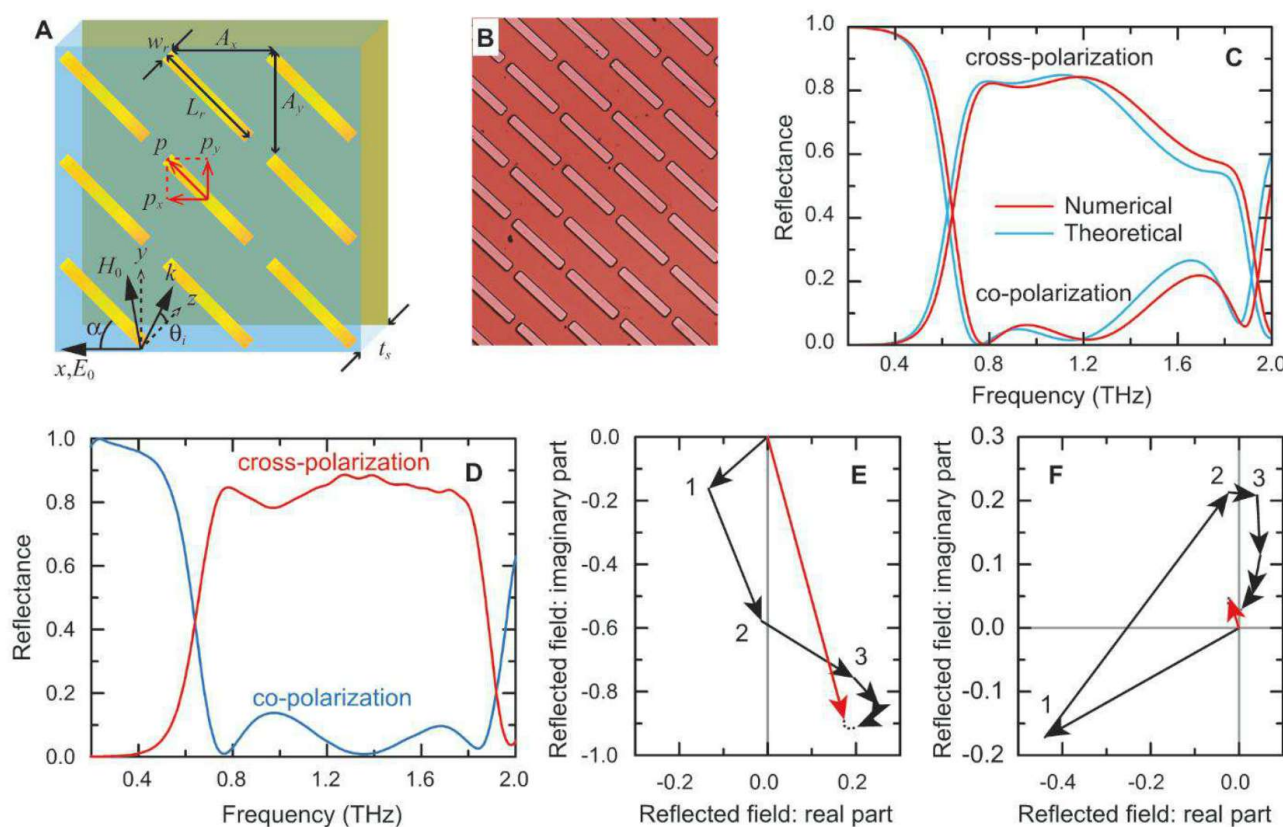


Fig. 1. Broadband polarization conversion in reflection. (A) Schematic and (B) optical micrograph of the metamaterial linear polarization converter. Both the gold cut-wire array and the gold ground plane are 200 nm thick, and they are separated by a polyimide dielectric spacer with thickness $t_s = 33 \mu\text{m}$ and dielectric constant $\epsilon = 3(1 + 0.05i)$. The periodicity $A_x = A_y = 68 \mu\text{m}$, cut-wire length $L_r = 82 \mu\text{m}$, and width $w_r = 10 \mu\text{m}$. The incidence angle $\theta_i = 25^\circ$, and the incident electric field $\mathbf{E}_0$ is linearly polarized in the x direction (s -polarized), with

an angle $\alpha = 45^\circ$ with respect to the cut-wire orientation. (C) Numerically simulated and theoretically calculated, and (D) experimentally measured co- and cross-polarized reflectance. (E) Cross- and (F) copolarized multiple reflections theoretically calculated at 0.76 THz, revealing the constructive and destructive interferences, respectively. Similar behavior occurs for other frequencies as well. The numbers j (1, 2, and 3) indicate the $(j - 1)$ -th roundtrip within the device. The red arrows are the converged cross- and copolarized reflected fields.

optical regime by using gold helix structures (17) and stacked nanorod arrays with a tailored rotational twist (18). Metamaterial-based polarimetric devices are particularly attractive in the terahertz frequency range due to the lack of suitable natural materials for terahertz device applications. However, the currently available designs suffer from either very limited bandwidth or high losses (19–21). In this work, we demonstrate high-efficiency and broadband, linear, terahertz polarization conversion using ultrathin planar metamaterials. In addition, our designs enable a dramatic improvement of the recently demonstrated anomalous (or generalized laws of) reflection/refraction (22, 23) by eliminating the ordinary components.

Our first metamaterial linear polarization converter design (Fig. 1, A and B) operates in reflection and consists of a metal cut-wire array and a metal ground plane separated by a dielectric spacer. We consider an incident wave E_0 linearly polarized in the x direction. It excites a dipolar oscillation p mainly along the cut-wires, which has parallel (p_x) and perpendicular (p_y) components to E_0 . Whereas E_0 and p_x determine the copolarized scattered field, p_y results in cross-polarized scattering, forming the dispersive reflection and

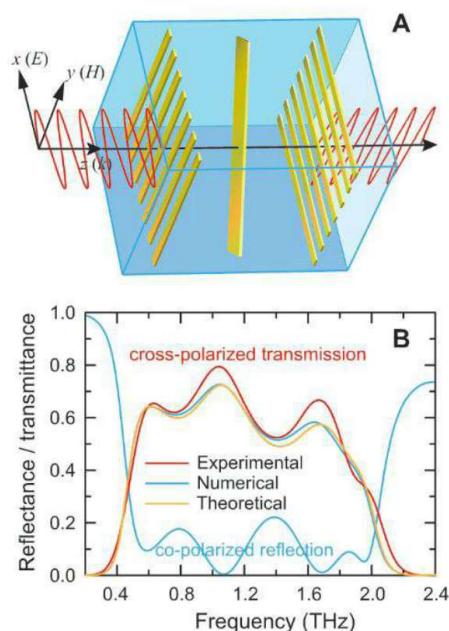


Fig. 2. Broadband polarization conversion in transmission. (A) Schematic of the unit cell of the metamaterial linear polarization converter, in which a normally incident x -polarized wave is converted into a y -polarized one. The cut-wire array is the same as in Fig. 1, the spacer is polyimide, and the separation between the cut-wire array and the gratings is $33\ \mu\text{m}$. The gold grating wire width is $4\ \mu\text{m}$, periodicity is $10\ \mu\text{m}$, and thickness is $200\ \text{nm}$. For this freestanding device, the gratings are covered with $4\text{-}\mu\text{m}$ -thick polyimide caps. (B) Cross-polarized transmittance obtained through experimental measurements, numerical simulations, and theoretical calculations. Also shown is the numerically simulated copolarized reflectance.

transmission (fig. S1) of the cut-wire array. Without the ground plane, the polarization conversion efficiency is low, and the overall reflection and transmission are elliptically polarized. The ground plane and the cut-wire array form a Fabry-Pérot-like cavity (24, 25); the consequent interference of polarization couplings in the multireflection process may either enhance or reduce the overall reflected fields with co- and cross-polarizations (26).

We validated this concept by performing full-wave numerical simulations (Fig. 1C) (the polarization angle dependence is available in fig. S2). Between 0.7 and 1.9 THz, the cross-polarized reflection carries more than 50% of the incident power, and the copolarized component is mostly below 20%. Between 0.8 and 1.36 THz, the cross-polarized reflection is higher than 80%, and the copolarized one is below 5%, representing a broadband and high-performance linear polarization converter in reflection. Further numerical simulations revealed that this broadband and high-efficiency performance is sustained over a wide incidence angle range (fig. S3). The broadband operation results from the superposition of multiple polarization conversion peaks around 0.8, 1.2, and 1.9 THz (Fig. 1C), at which the efficiency is mainly limited by dielectric loss. At other frequencies, the copolarized reflection also contributes.

Our fabricated devices are characterized by using terahertz time-domain spectroscopy (THz-TDS) (fig. S4), as detailed in (26). The experimental co- and cross-polarized reflectance (Fig. 1D) reproduces the linear polarization conversion seen in the numerical simulations. From 0.65 to 1.87 THz, the cross-polarized reflection relays more than 50% of the incident power and over 80% between 0.73 and 1.8 THz, with the highest conversion efficiency of 88% at 1.36 THz. The copolarized reflectance is mostly less than 14% and approaches zero at some frequencies, demonstrating the capability of rotating the input linear polarization by 90° with high output purity over a broad bandwidth.

The underlying reason for the enhanced polarization conversion is the interference between the multiple polarization couplings in the Fabry-Pérot-like cavity. To confirm this interpretation, we calculated the co- and cross-polarized reflected fields caused by each roundtrip within the cavity (fig. S5). For each individual reflection, we plotted the complex reflected field in Fig. 1, E and F. As expected, the superposition of these partial cross-polarized reflected fields results in a constructive interference and gives a near-unity overall cross-reflection (Fig. 1E, red arrow); the superposition of these partial copolarized reflected fields results in a destructive interference and gives a zero overall coreflection (Fig. 1F, red arrow). The calculated overall reflections (Fig. 1C) are in excellent agreement with both the numerical simulations and experimental data (26).

Many applications require linear polarization conversion in transmission mode, for which the metal ground plane must be replaced. Our solution is to use a metal grating that transmits the

cross-polarized waves while still acting as a ground plane for copolarized waves. To retain the backward-propagating cross-polarized waves without blocking the incident waves, we added an orthogonal metal grating in front of the cut-wire (Fig. 2A). In addition, we added a $4\text{-}\mu\text{m}$ -thick polyimide capping layer both before the front and behind the back grating. This ultrathin, freestanding device serves as a high-performance linear polarization converter in transmission with reduced co- and cross-polarized reflections. In Fig. 2B, we plotted the numerically simulated cross-polarized transmittance and copolarized reflectance, for normal incidence with an x -polarized incident electric field [this device also operates over a wide incidence angle range (fig. S6)]. Also shown in Fig. 2B are the theoretical multireflection model results (26) and measured data for our fabricated device. There is excellent agreement among the numerical, experimental, and theoretical results. The device is able to rotate the linear polarization by 90° , with a conversion efficiency exceeding 50% from 0.52 to 1.82 THz, with the highest efficiency of 80% at 1.04 THz. In both simulation and experiments, the copolarized transmission and cross-polarized reflection are practically zero because of the use of gratings. The measured ratio between the co- and cross-polarized transmittance is less than 0.1 between 0.2 and 2.2 THz, covering the whole frequency range in a typical THz-TDS. The device performance, limited by the dielectric loss and copolarized reflection (Fig. 2B), can be further improved through optimizing the structural design and using lower-loss dielectric materials.

Recent demonstrations of the general laws of reflection/refraction and wavefront-shaping (22, 23) rely on creating a phase gradient in the cross-polarized scattering from anisotropic metamaterials. However, the single-layered metamaterial only produced weak anomalously reflected/refracted beams, with most of the power remaining in the ordinary beams. Our high-efficiency linear polarization converters allow us to accomplish broadband, near-perfect anomalous reflection/refraction by largely eliminating the ordinary beams. We used eight anisotropic resonators with various geometries and dimensions in a super-unit-cell (Fig. 3, A and B) to create a linear phase variation of the cross-polarized transmission spanning a 2π range. The resonator dimensions were determined through numerical simulations and are specified in fig. S7. Each of these resonators can be used to construct a high-performance linear polarization converter with similar cross-polarized transmission but a phase increment of $\sim\pi/4$ (Fig. 3C). Therefore, when combined into the super-unit-cell shown in Fig. 3, A and B, we expect a linear phase gradient of the cross-polarized transmitted wavefront, resulting in anomalous refraction.

We characterize our fabricated free-standing sample under normal incidence ($\theta_i = 0$) (26). In Fig. 3D, we plotted the cross-polarized transmittance as a function of frequency and transmission angle (the measured co-polarized transmission is negligible). Over a broad bandwidth, the ordinary

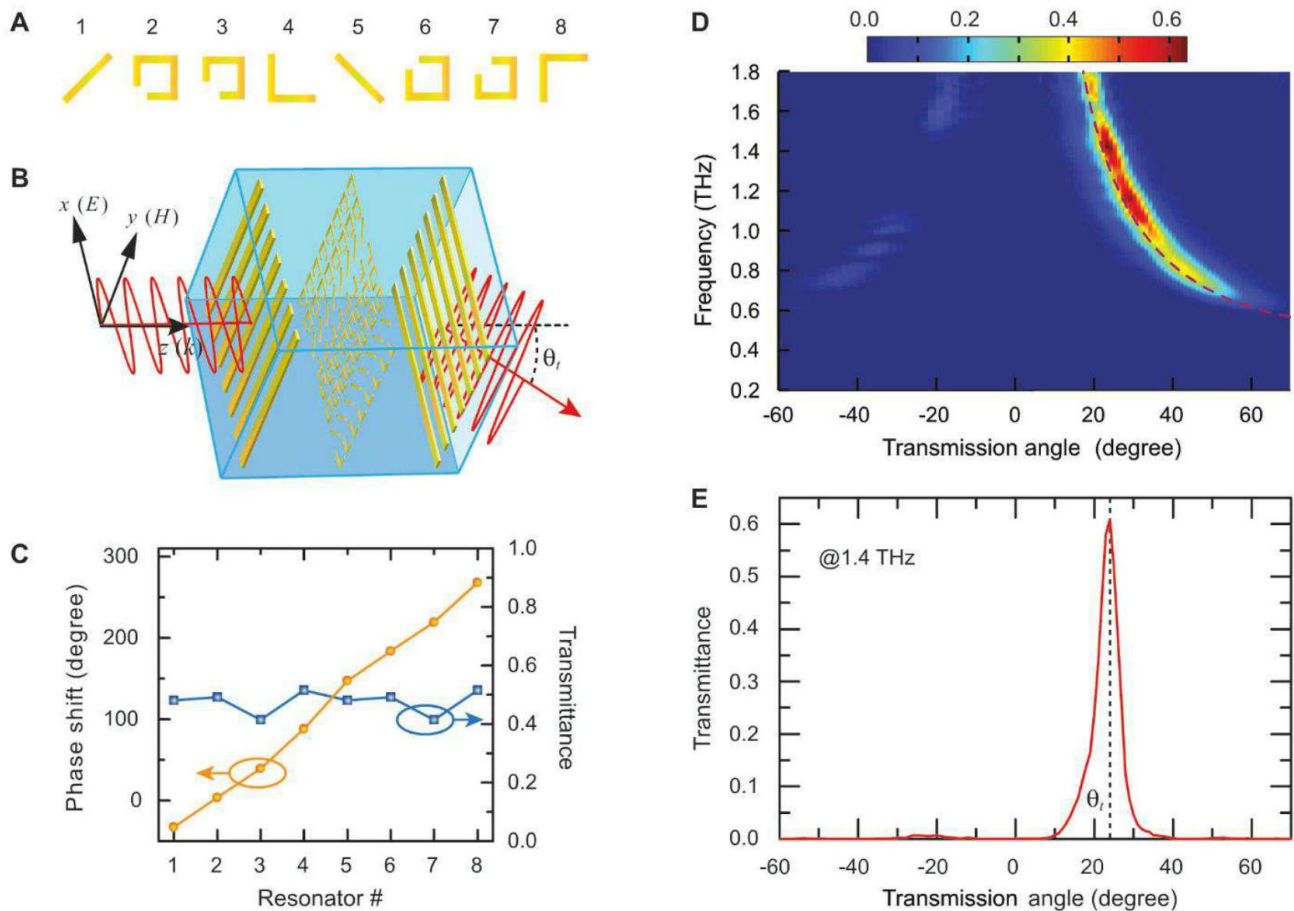


Fig. 3. Broadband and near-perfect anomalous refraction. (A) Resonator array super-unit-cell within the (B) anomalous refraction design (not to scale). A normally incident x -polarized wave is converted to a y -polarized transmission beam, which bends in the x - z plane to an angle θ_t with respect to the z axis. (C) The simulated cross-polarized transmittance and corresponding phase

shift when each individual resonator is used in the linear polarization converter. (D) Experimentally measured cross-polarized transmittance as a function of frequency and angle. The dashed curve is the theoretically calculated frequency-dependent bending angle. (E) Cross-polarized transmittance at 1.4 THz under normal incidence as a function of angle.

refraction ($\theta_t = 0$) is practically zero, and only the anomalous beam is transmitted at a frequency-dependent refraction angle θ_t following the generalized law of refraction (22): $n_1 \sin(\theta_i) - n_2 \sin(\theta_t) = d\Phi/dx$, where $n_1 = n_2 = 1$ for the surrounding air, $d\Phi/dx = \lambda_0/D_1$ is the phase gradient imposed by the sample, λ_0 is the free space wavelength, and $D_1 = 560 \mu\text{m}$ is the periodicity of the super-unit-cell along the phase gradient (x direction). The calculated frequency-dependent anomalous refraction angle is plotted as the dashed curve in Fig. 3D, revealing excellent agreement with the experimental results. At 1.4 THz ($\lambda_0 = 214 \mu\text{m}$), the angle-dependent transmittance (Fig. 3E) shows a maximum output power of 61% at $\theta_t = 24^\circ$ and reveals minimal transmission at negative angles (0.6% at -24°). Also, the anomalous refraction intensity drops to zero as λ_0 approaches the periodicity D_1 corresponding to 0.54 THz, at which θ_t approaches 90° (Fig. 3D). This indicates that the incident wave is either totally reflected or converted to surface waves (27).

Our devices are ultrathin and operate within the technologically relevant terahertz frequency range, in which many important functionalities—

including polarization conversion, beam steering, and wavefront shaping—have been extremely challenging to accomplish. Our results have shown that we can achieve broadband, high-performance linear polarization conversion and near-perfect anomalous refraction. Additional numerical simulations show that near-perfect anomalous reflection can also be accomplished by using the same concept (fig. S8). No particular optimization was undertaken to increase the conversion efficiency and bandwidth. Our demonstrations can be extended to other relevant frequencies. However, fabrication challenges and metal losses can become issues when approaching visible frequencies that substantially degrade the device performance. Our results form the foundation for more advanced applications; for instance, an appropriately constructed device can serve as a high-performance spatial light modulator. The wavefront shaping can result in a helical phase dependence forming Laguerre-Gauss modes carrying an orbital angular momentum that can acquire any integer value (28), which is useful in quantum entanglement (29) and enables opportunities in telecommunications (30).

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Short-Range Quantum Magnetism of Ultracold Fermions in an Optical Lattice

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Quantum magnetism originates from the exchange coupling between quantum mechanical spins. Here, we report on the observation of nearest-neighbor magnetic correlations emerging in the many-body state of a thermalized Fermi gas in an optical lattice. The key to obtaining short-range magnetic order is a local redistribution of entropy, which allows temperatures below the exchange energy for a subset of lattice bonds. When loading a repulsively interacting gas into either dimerized or anisotropic simple cubic configurations of a tunable-geometry lattice, we observe an excess of singlets as compared with triplets consisting of two opposite spins. For the anisotropic lattice, the transverse spin correlator reveals antiferromagnetic correlations along one spatial axis. Our work facilitates addressing open problems in quantum magnetism through the use of quantum simulation.

Quantum magnetism describes quantum many-body states of spins coupled by exchange interactions and lies at the heart of many fundamental phenomena in condensed matter physics (1, 2). Spin systems often tend to show long-range order at low temperatures; however, the interplay of exchange interactions with geometry and quantum fluctuations can lead to quantum states characterized by their short-range magnetic order. Examples include valence-bond crystals, spin-liquids, and possibly high-temperature superconductors (3–5). Interestingly, the underlying many-body physics gives rise to computationally and theoretically intractable regimes, even in the phase diagrams of simple models such as the Fermi-Hubbard model. Moreover, the direct measurement of local spin correlations in solids remains a major challenge.

The controlled setting of ultracold fermionic atoms in optical lattices is regarded as a promising route to gain new insights into phases driven by quantum magnetism (6–8). This approach offers experimental access to a clean and highly

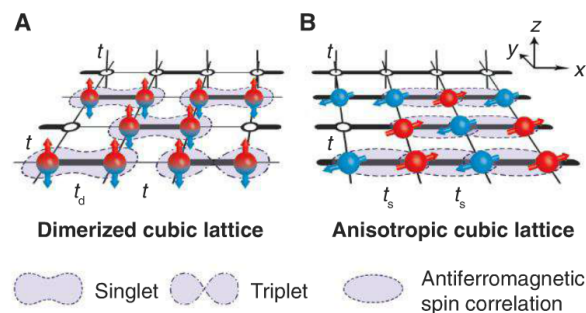
flexible Fermi-Hubbard model with a distinct set of observables (9). For repulsively interacting fermions, density ordering in the metal–Mott insulator transition has been explored experimentally (10, 11). Yet, progress toward entering the regime of quantum magnetism has been hindered by the ultralow temperatures and entropies required to observe exchange-driven spin ordering in optical lattices. For bosonic systems, promising progress has been reported: By mapping the spin onto the site occupation or the local phase of a Bose-Einstein condensate, the physics of one-dimensional (1D) decoupled Ising spin chains (12) and classical magnetism on a

triangular lattice could be simulated (13). Furthermore, arrays of isolated double wells and plaquettes were used to study the exchange dynamics of artificially prepared few-boson systems (14, 15).

To enable the study of quantum magnetic phenomena in thermalized many-body Hubbard systems, cooling schemes based on the redistribution of entropy between different regions of the trap have been suggested (16, 17). In this work, we propose and implement a local entropy-redistribution scheme within the lattice structure to reach the regime of quantum magnetism. The atoms are either prepared in a dimerized or an anisotropic simple cubic lattice (Fig. 1). In both geometries, a subset of links of the underlying simple cubic lattice is set to a larger exchange energy as compared with the other links. As a result, the entropy is predominantly stored in configurations involving the weak links. For fixed total entropy in the trapped system, this allows us to reach temperatures between the two exchange-energy scales.

The experiment is performed with a harmonically confined, balanced two-component mixture of a quantum degenerate Fermi gas of ⁴⁰K. The atoms are prepared in two magnetic sublevels, $m_F = -9/2$ and $-7/2$, of the $F = 9/2$ hyperfine manifold, denoted by $\uparrow$ and $\downarrow$, at temperatures below 10% of the Fermi temperature. We load 50,000 to 100,000 repulsively interacting atoms at an s-wave scattering length of $106(1)a_0$ into the 3D optical lattice, where a_0 denotes the Bohr radius. The lattice is created by a combination of interfering laser beams

Fig. 1. Magnetic spin correlations. Schematic view of the nearest-neighbor spin correlations observed in the experiment. A two-component mixture of fermionic atoms (red and blue) is prepared close to half-filling in a cubic lattice with two different tunnel-coupling configurations. (A) Dimerized lattice with the strong dimer links t_d and weaker links t . Low temperatures lead to an excess number of singlets over triplets. (B) Anisotropic lattice with strong and weak tunneling t_s and t along different spatial axes. Antiferromagnetic spin correlations in the transverse direction are formed along the strong-link direction. In both panels, exemplary thermal excitations in the form of spin excitations, or holes, are shown.



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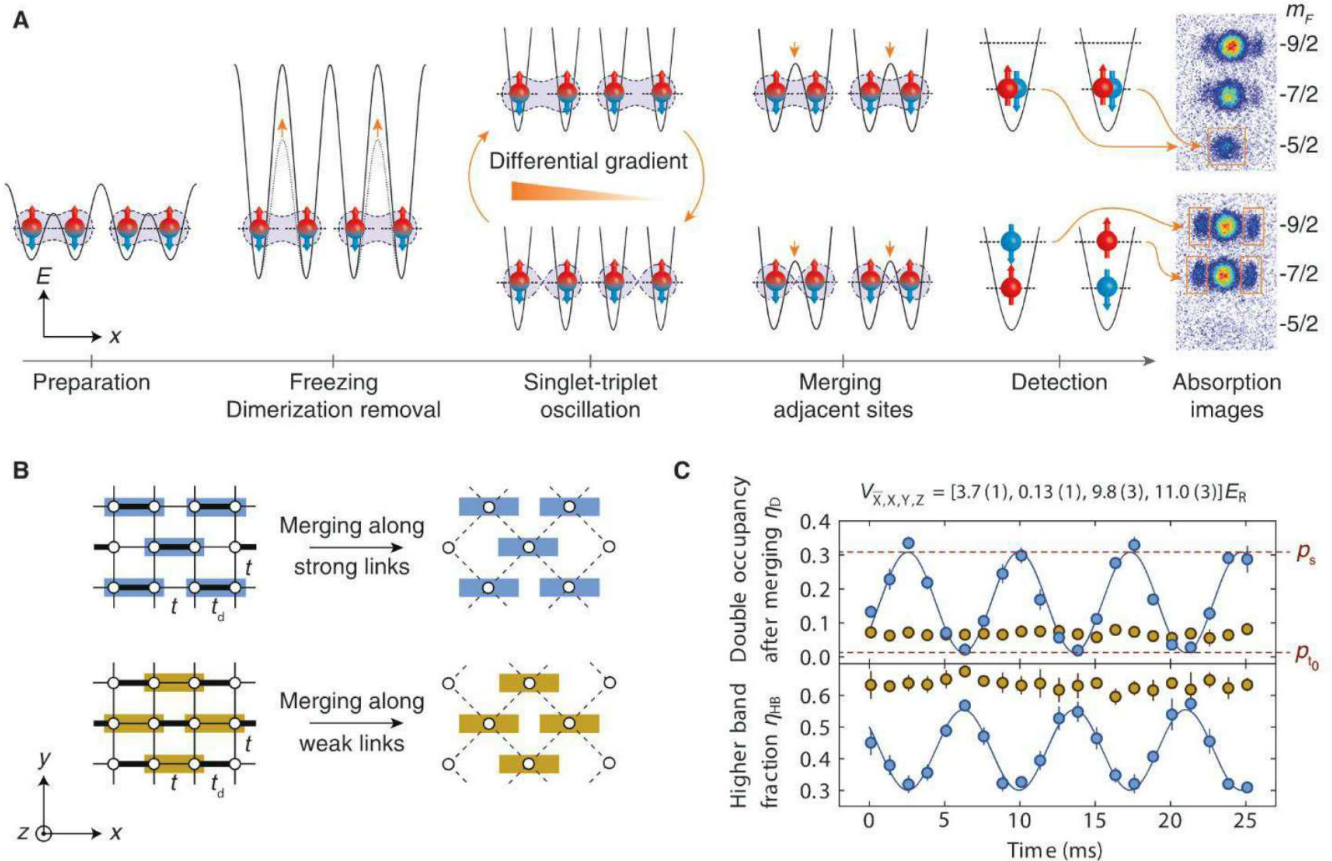


Fig. 2. Detection scheme for singlets and triplets. (A) Schematics of the detection steps for the case of two singlets in a dimerized lattice. Depending on the oscillation time, the absorption images on the right show either a large double occupancy in the lowest band, corresponding to singlets (top row), or an increased higher-band fraction, indicating triplet states (bottom row). (B) The two possible merging configurations in a dimerized lattice. Singlets and triplets are detected on a set of adjacent sites arranged on a checkerboard pattern in the plane. (C)

Singlet-triplet oscillation in a strongly dimerized lattice with $U/t = 10.9(8)$ and $t_d/t = 20(2)$. We observe oscillations in the double occupancy after merging, η_D , and in the higher-band fraction, η_{HB} , when merging along the strong links (blue), whereas no oscillations are visible for the weak links (ocher). The phase of the oscillation is shifted, owing to the double-occupancy-removal procedure (20). The red dashed lines denote the extracted singlet and triplet fractions, p_s and p_{t0} , respectively. Error bars indicate SD of at least five measurements.

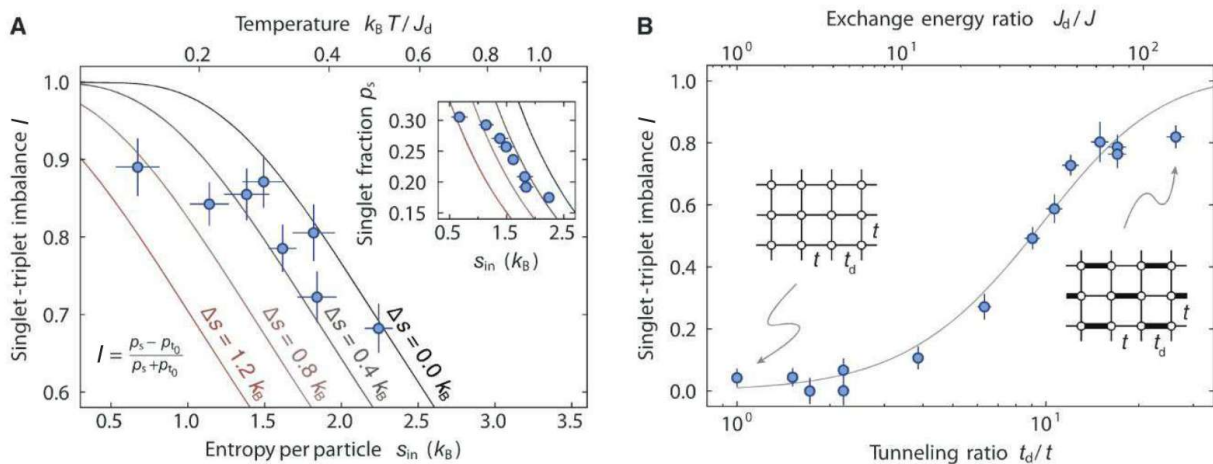


Fig. 3. Dimerized simple cubic lattice. (A) Singlet-triplet imbalance on the strong dimer links versus initial entropy before loading into the lattice (s_{in}) and temperature ($k_B T/J_d$) in a dimerized lattice with $U/t = 10.9(8)$ and $t_d/t = 20(2)$, where $t/h = 67(6)$ Hz. This corresponds to an exchange-energy ratio of $J_d/J = 100(9)$, with $J/h = 24(2)$ Hz. The imbalance and absolute singlet fraction (inset) decrease with increasing entropy. Solid curves show the prediction of a high-temperature series expansion, taking into account different amounts of added entropy Δs during the lattice loading. (B) Imbalance versus dimerization t_d/t

and J_d/J , showing an increase for strongly dimerized simple cubic lattices. As the dimerization is increased, the exchange energy J/h increases from $18(3)$ to $27(3)$ Hz, because U/t is reduced (20). The solid curve shows the theory prediction for an entropy per particle of $1.7 k_B$ in the lattice, which includes the heating during loading. Vertical error bars denote the fit error from singlet-triplet oscillations consisting of 63 measurements, the errors in t_d/t stem from lattice calibration uncertainties, and the errors in s_{in} are the SD of five measurements. Individual curves of p_s and p_{t0} for all measurements are shown in (20).

operating at a wavelength $\lambda = 1064$ nm with lattice depths $V_{\bar{x}}$, V_x , V_y , and V_z , which are given in units of recoil energy $E_R = \hbar^2/2m\lambda^2$ (18–20). Here, m denotes the mass of ^{40}K and $\hbar$ is Planck's constant. We independently control the tunneling strengths along all three spatial axes. In addition, we can introduce a checkerboard dimerization in the xy plane by strengthening every second tunneling link along the x axis (Fig. 1A). The checkerboard pattern is replicated along the z axis. Our system is well described by a 3D single-band Hubbard model with repulsive on-site interaction energy U , unless explicitly stated. The tunneling t along the weak links in both lattice geometries is set to $t/\hbar = 67(6)$ Hz for all measurements. The strong tunneling is denoted by t_d and t_s in the dimerized and anisotropic lattice, respectively.

As shown in Fig. 2, A and B, the fraction of atoms forming singlets (p_s) and triplets (p_t) on neighboring lattice sites is detected by transforming the lattice to a checkerboard geometry, similar to a previously developed technique (21). The first part of our detection sequence consists of freezing out the atomic motion. In the anisotropic lattice, we perform a sudden ramp into a deep, simple cubic structure with negligible tunneling. The final singlet measurement then corresponds to projecting pairs of sites onto $|s\rangle = |\uparrow, \downarrow\rangle - |\downarrow, \uparrow\rangle/\sqrt{2}$. In the dimerized lattice, we apply a two-step ramp, such that the final singlet measurement locally projects onto the singlet eigenstate of an isolated pair of sites (20). There, the intensity of all lattice beams is ramped up proportionally, before the lattice is adiabatically transformed into a simple cubic configuration. For both lattice geometries, the final

triplet measurement projects pairs of sites onto $|t_0\rangle = |\uparrow, \downarrow\rangle + |\downarrow, \uparrow\rangle/\sqrt{2}$. In the deep cubic lattice, all atoms on doubly occupied sites are removed. We then apply a magnetic field gradient, which creates a differential bias energy Δ for atoms of opposite spins on adjacent sites and causes coherent oscillations between the singlet $|s\rangle$ and triplet $|t_0\rangle$ states at a frequency $\nu = \Delta/\hbar$. If the initial amount of singlets and triplets is equal, no overall oscillation will be visible, as $|s\rangle$ and $|t_0\rangle$ oscillate in antiphase.

After a certain oscillation time, we remove the gradient and merge pairs of adjacent sites. Owing to the symmetry of the two-particle wave function, the singlet state on neighboring sites evolves to a doubly occupied site with both atoms in the lowest band, whereas the triplet state transforms into a state with one atom in the lowest and one atom in the first excited band. The fraction of atoms forming double occupancies in the lowest band of the merged lattice, η_D , is detected by an interaction-dependent radiofrequency transfer to the previously unpopulated $m_F = -5/2$ spin state (10). The fraction of atoms in the higher band, η_{HB} , is obtained from a band-mapping technique (8). For the final readout, we take absorption images after Stern-Gerlach separation of the spin states during ballistic expansion. For an imbalance between the initial singlet and triplet populations, η_D and η_{HB} will show oscillations with opposite amplitudes. As the double occupancy in the lowest band contains only contributions from two particles with opposite spins, we can infer the fraction of atoms forming singlets and triplets from the maxima and minima of a sinusoidal fit to η_D . The higher-band fraction has an additional offset caused by dimers containing two atoms

with the same spin or one atom with an anti-symmetric spatial wave function.

When loading atoms into a strongly dimerized lattice and merging along the strong links, we observe oscillations in η_D and η_{HB} (Fig. 2C). This reveals an excess number of singlets, corresponding to magnetic order on neighboring sites. We quantify this order by the normalized imbalance

$$I = \frac{p_s - p_t}{p_s + p_t} \quad (1)$$

The order in the strongly dimerized lattice originates from temperatures below the intradimer exchange energy $J_d = -U/2 + \sqrt{16t_d^2 + U^2}/2$, which denotes the singlet-triplet splitting on a single dimer. Although such temperatures require very low entropies for isotropic lattices (22), in our system the access to the regime of magnetic ordering is facilitated by the presence of the weaker interdimer exchange energy $J \ll J_d$. This leads to an entropy redistribution from states on the strong to the weak links and gives access to the temperature regime $J < k_B T < J_d$ for experimentally attainable entropies (here, k_B denotes the Boltzmann constant and T is temperature). As expected for strong dimerizations, we find no visible oscillations when merging along the weak links, which indicates the absence of magnetic correlations on these links (Fig. 2C). We measure constant values of $\eta_D = 0.07(1)$ and $\eta_{HB} = 0.63(3)$, which are consistent with a state in which nearly all singlets are located on neighboring dimer links, with vanishing correlations between them. The observed correlation pattern

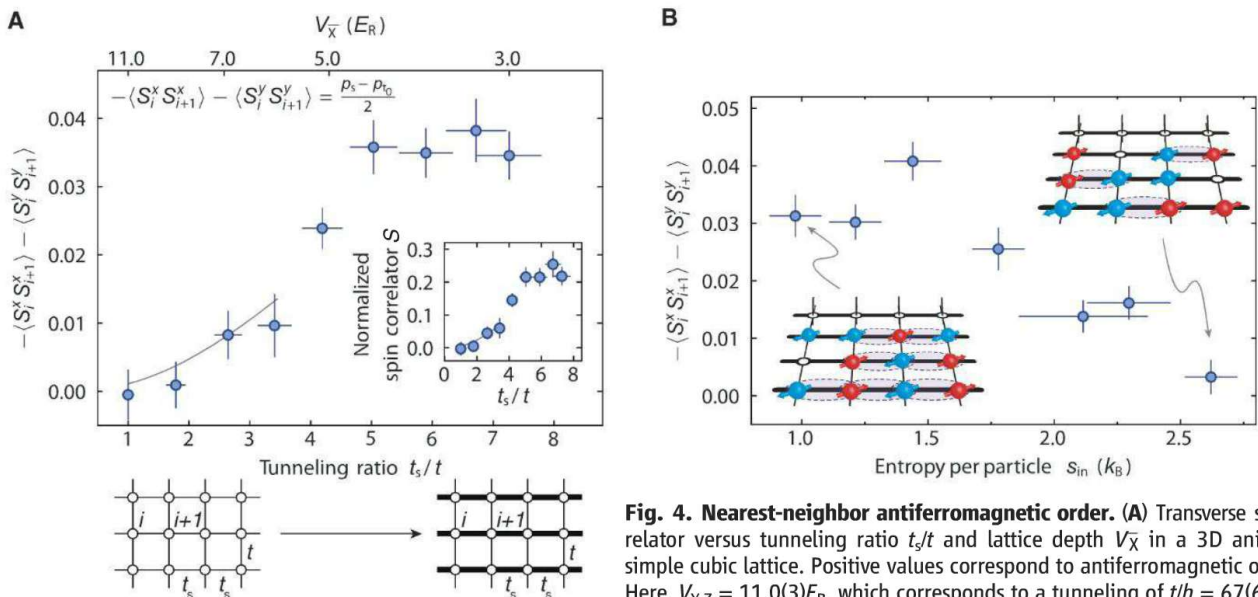


Fig. 4. Nearest-neighbor antiferromagnetic order. (A) Transverse spin correlator versus tunneling ratio t_s/t and lattice depth $V_{\bar{x}}$ in a 3D anisotropic simple cubic lattice. Positive values correspond to antiferromagnetic ordering. Here, $V_{y,z} = 11.0(3)E_R$, which corresponds to a tunneling of $t/\hbar = 67(6)$ Hz. As the tunneling ratio is increased, the exchange-energy ratio J_s/J increases from 1.0(2) to 27(4). At the same time, U/t is reduced (20), leading to an increase of the weak exchange $J/\hbar$ from 16(3) to 37(5) Hz. The inset shows the normalized spin correlator S quantifying the amount of antiferromagnetic ordering at the relevant density. Solid curves are the prediction of a high-temperature series expansion for an entropy-per-particle of $1.7k_B$ (as used in Fig. 3B) and are shown up to $t_s/k_B T = 1/2$. (B) Transverse spin correlator versus entropy before loading into the lattice at $U/t = 10.5(8)$ and $t_s/t = 7.3(6)$, together with a schematic view of the spin ordering. Error bars are as in Fig. 3.

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closely resembles that of an explicit valence-bond solid (3).

To analyze the effect of temperature on the magnetic correlations, we measure the dependence of the singlet-triplet imbalance on the entropy in the harmonic trap s_{in} (Fig. 3A). The imbalance I and the absolute singlet fraction p_s reduce for larger entropies, as triplet states become thermally populated. The singlet fraction is additionally diminished by a shrinking half-filled region in the trapped system (23). We find good agreement with a second-order, high-temperature series expansion of coupled dimers when including an entropy increase of $\Delta s = 0.4k_B$, which is associated with the lattice loading (24); Δs is larger for the lowest entropies, consistent with previous results (23). From the measured imbalances we infer temperatures below $0.4J_d$, which corresponds to a trap-averaged entropy per particle of $1.6k_B$. Owing to entropy redistribution in the trap, the entropy per particle in the half-filled region drops below $0.6k_B$, which is 20% lower than for the isotropic simple cubic lattice [see (20)]. This reduction in entropy is even more pronounced for lower total entropies; in the absence of heating, this would result in entropies below $0.1k_B$ in the trap center.

For reduced dimerizations, the coupling between dimers leads to increased interdimer correlations. The excitation energy of triplets is then lowered as they delocalize over the lattice, thus changing the nature of magnetic ordering. In Fig. 3B, we use the tunable lattice to investigate the dependence of the imbalance I on the tunneling ratio t_d/t . In good agreement with theory, the imbalance decreases and eventually falls below our experimental resolution as the dimerization is progressively removed. This decrease can be attributed to the intradimer exchange energy J_d becoming smaller than the temperature T . For vanishing temperatures, the system is expected to undergo a quantum phase transition from a gapped spin-liquid state to a long-range-ordered antiferromagnet as t_d/t is reduced below a critical value, where the spin gap becomes zero (2, 25). In the isotropic simple cubic lattice ($t_d/t = 1$), the system is in the metal-to-Mott insulator crossover regime with $U/t = 16(1)$ and a calculated hole probability of $\sim 15\%$ in the half-filled region (20).

The key to the observation of quantum magnetism in our system is the presence of two different exchange-energy scales. Without dimerization, this situation also occurs for anisotropic simple cubic lattices with tunneling t along two axes and a stronger tunneling t_s along the third direction. In this case, the symmetry between neighboring links is restored, and the detected singlet and triplet fractions are the same for both merging configurations. We observe a clear population difference $(p_s - p_{t_0})/2$ after loading a gas with entropies s_{in} below $1.0k_B$ into an anisotropic lattice, which increases to 4% for larger tunneling ratios t_s/t (Fig. 4A). The population difference is equal to the transverse spin correlator between neighbor-

ing sites i and $i + 1$ along the strong tunneling direction

$$-\langle S_i^x S_{i+1}^x \rangle - \langle S_i^y S_{i+1}^y \rangle = (p_s - p_{t_0})/2 \quad (2)$$

Hence, this quantity directly characterizes the fraction of atoms with antiferromagnetic ordering on neighboring sites in the entire atomic cloud. Our observations also extend to weak lattices, where correction terms to the single-band Hubbard model become relevant (26). In this regime, a variety of magnetic phases have been predicted (27, 28).

The results can be compared to a second-order, high-temperature series expansion (20). We find good agreement in the regime of small anisotropies. For larger anisotropies, the expansion breaks down as the strong tunneling and the temperature become comparable. In this regime, we expect the temperature to lie between the weak and strong exchange scales $J < k_B T < J_s$. The system then behaves as an array of 1D spin-ordered chains without correlations between them (29), where the majority of the entropy is stored in configurations involving the weak links. Low-dimensional systems have been predicted to show enhanced nearest-neighbor correlations (30).

For temperatures much larger than J_s , magnetic correlations should disappear. In Fig. 4B, we show the dependence on the initial entropy s_{in} and find the correlations to vanish above $2.5k_B$, where $k_B T \gg J_s$ is expected.

Owing to the presence of the harmonic trap, most spin-correlated atoms are located in the center, where the filling is close to one particle per site. The density-normalized fraction of antiferromagnetic ordering is obtained when dividing by the fraction of atoms with two particles of arbitrary spin on adjacent sites. Under the assumption that all spin correlators $\langle S_i^{x,y,z} S_{i+1}^{x,y,z} \rangle$ are equal, which applies if all symmetry-breaking fields are much smaller than all other energy scales, the normalized spin correlator S can be directly obtained from the measurement of singlets and triplets (here, n_i^s is 1 for a single particle of any spin on site i and 0 otherwise)

$$S = \frac{-4\langle S_i^z S_{i+1}^z \rangle}{\langle n_i^s n_{i+1}^s \rangle} = \frac{p_s - p_{t_0}}{p_s + 3p_{t_0}} \quad (3)$$

The normalized antiferromagnetic correlations along the strong tunneling direction reach 25% (Fig. 4A, inset), which corresponds to ~ 5000 ordered atoms.

Our local entropy redistribution scheme can be generalized to access the low-temperature regime in different geometries; for example, in weakly coupled 2D systems. Further extensions include the isolation of low-entropy regions via repulsive potentials, introducing additional lattice layers at different fillings as entropy reservoirs, or enhancing the tunnel coupling only in the central part of the atomic cloud. The tunable-geometry

optical lattice allows the extension of our studies to spin-ladder systems, dimerized 1D chains, and zigzag chains, where the interplay between quantum fluctuations and magnetic ordering plays a particularly important role (3, 29, 31). At even lower temperatures, the existence of spin-liquids in honeycomb or triangular lattices could be investigated (32).

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S6

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Strong Light-Matter Interactions in Heterostructures of Atomically Thin Films

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The isolation of various two-dimensional (2D) materials, and the possibility to combine them in vertical stacks, has created a new paradigm in materials science: heterostructures based on 2D crystals. Such a concept has already proven fruitful for a number of electronic applications in the area of ultrathin and flexible devices. Here, we expand the range of such structures to photoactive ones by using semiconducting transition metal dichalcogenides (TMDCs)/graphene stacks. Van Hove singularities in the electronic density of states of TMDC guarantees enhanced light-matter interactions, leading to enhanced photon absorption and electron-hole creation (which are collected in transparent graphene electrodes). This allows development of extremely efficient flexible photovoltaic devices with photoresponsivity above 0.1 ampere per watt (corresponding to an external quantum efficiency of above 30%).

The advent of graphene (1) and the subsequent discovery of its multitude of superior properties (2–5) has led to the identification of many other two-dimensional (2D) crystals (6) through both chemical modification of graphene and exfoliation of other layered compounds. This new area of research and progress in precise transfer of the crystals while maintaining their quality (7, 8) has resulted in the emergence of a new class of materials: heterostructures based on 2D atomic crystals (5, 8, 9). More specifically, there is the possibility to create hybrid materials by stacking combinations of 2D crystals with differing properties. These structures are interesting from both fundamental and application points of view. It has, for instance, been shown that layering sheets of graphene and hexagonal boron nitride (hBN), molybdenum disulfide (MoS₂), or tungsten disulfide (WS₂) allows operation of tunneling transistors (9, 10) and permitted the observation of phenomena such as Coulomb drag (11) and the fractional quantum Hall effect (12).

Many other crystals have been found to exfoliate to monolayer by both mechanical (6) and chemical methods (13). Transition metal dichalcogenides (TMDCs) are a group of layered ma-

terials that has attracted a lot of interest (14). They are structured such that each layer consists of three atomic planes: a triangular lattice of transition metal atoms sandwiched between two triangular lattices of chalcogen atoms (S, Se, or Te). There is strong covalent bonding between the atoms within each layer and predominantly weak

van der Waals bonding between adjacent layers. Many of these materials—NbSe₂, MoS₂, WS₂, and TaS₂, to name a few—are structurally similar but have an array of electronic properties ranging from semiconducting (15) to metallic (16), from charge density waves to superconducting (17), depending on their exact composition, electronic density, geometry, and thickness (18).

Besides the traditional applications of TMDC films as solid-state lubricants and industrial surface protection (19, 20), films of these materials have long been considered for photovoltaic devices, due to their large optical absorption, which is greater than 10⁷ m⁻¹ across the visible range, meaning that 95% of the light can be absorbed by a 300-nm film. A further advantage of WS₂ is its chemical stability (21, 22) and band gaps in the visible part of the spectrum (22–24).

Previously, planar WS₂ (21) and MoS₂ (22) structures were studied for photovoltaic applications. However, efforts to extract photocurrent have been hampered by the need to create a p-n junction to separate the electron-hole (e-h) pairs created by incoming photons. Here, we show that, with the arrival of vertical 2D-crystal-based heterostructures, a beneficial combination of each material's properties emerges: TMDCs as good photoactive materials and graphene as a good transparent electrode. Using a Gr/TMDC/Gr stack (here, Gr stands for graphene) with appropriately

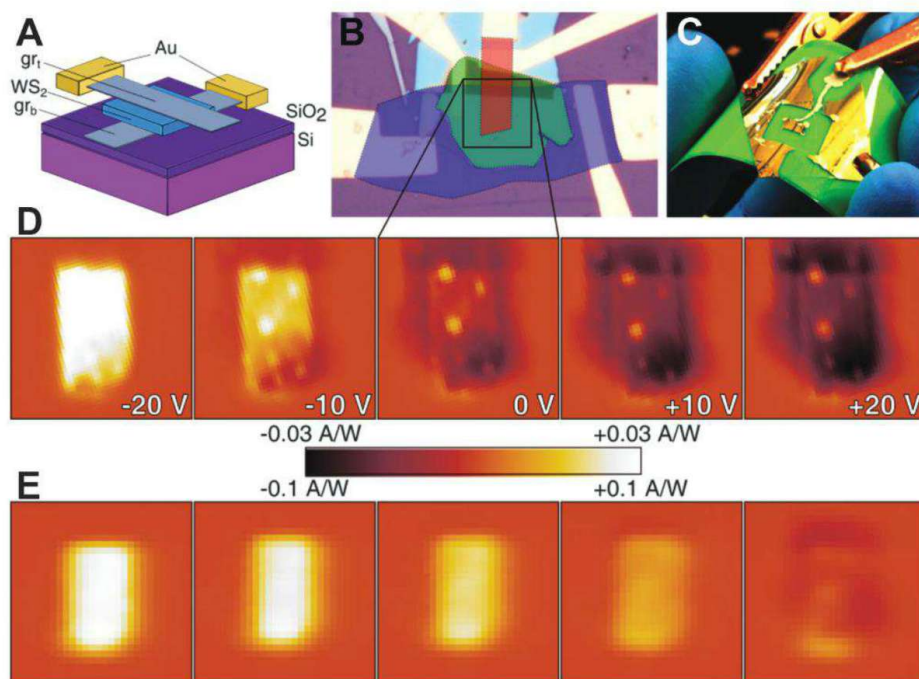


Fig. 1. Device structure and photocurrent mapping. (A) A schematic of the device with the principal layers shown. hBN is not shown. (B) An optical micrograph of one of our devices. The shading of the three constituent layers denotes the regions of the respective materials—top and bottom graphene electrodes are shown in red and blue, and WS₂ is shown in green. (C) A photograph of one of our flexible devices placed on an electroluminescent mat. (D and E) photocurrent maps taken before (D) and after (E) doping the top graphene layer with water vapor. A signal is only seen in the area where all three layers overlap. The two graphene layers were connected via a 1 k Ω resistor, on which the photocurrent was measured. No bias was applied, and for both (E) and (D), the maps were taken at gate voltages from -20 V to $+20$ V. The scale of the maps is given by their width, 20 μ m.

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positioned Fermi levels and simply doping the two graphene layers differently (either by electrostatic gating or chemical methods) leads to a large photocurrent. The layered nature of our structures and exceptional mechanical strength of graphene and TMDC crystals (25, 26) also allowed us to fabricate flexible devices. Without illumination, such devices act as tunneling transistors (9, 10).

Although we concentrate the experimental data on the properties of Gr/WS₂/Gr heterostructures, our results are generic for a large class of systems where semiconducting TMDCs are the key element [see (27) for other examples of Gr/TMDC/Gr heterostructures].

Our devices comprise three principal elements—top and bottom graphene electrode layers (both micromechanically cleaved and chemical vapor deposition (CVD)-grown graphene were tested), sandwiching a photoactive TMDC layer (Fig. 1). In the fabrication procedure, the flakes were transferred with the “dry transfer” technique (in the case of micromechanically cleaved graphene) (7, 8) with thorough annealing (27) at each stage to ensure minimal contamination between the layers (28) and low-level doping of the graphene layers. We also chose to use hBN as both a substrate and an encapsulating layer to achieve a higher doping homogeneity (7, 29). Thus, the final structure of a typical device, on top of an oxidized silicon wafer or flexible polyethylene terephthalate (PET) film, was hBN/Gr/WS₂/Gr/hBN. In the case of nonflexible devices on Si/SiO₂, the doped silicon could be used as a back gate and SiO₂/hBN (typically 300 nm of SiO₂ and 20 nm of hBN) can be used as the gate dielectric. A series of such structures was produced where the thickness of the TMDC layer was varied from ~5 to 50 nm.

The current-voltage (*I-V*) characteristics of our samples strongly depended on illumination (Fig. 2A, left axis). Without illumination, the devices displayed strongly nonlinear *I-V* curves (Fig. 2A, right axis). Comparing the two sets of *I-V* curves, there is strong contrast to when they were illuminated: The resistance drops by more than three orders of magnitude, and the curves are linear around zero bias. At higher bias ($\sim \pm 0.2$ V), the current saturates, as the number of available charge carriers in the photoactive region becomes limited.

The photocurrent generated in our devices was mapped by scanning photocurrent microscopy, where a laser spot was scanned over the sample, and the resultant photocurrent was displayed as a function of laser spot position. Photocurrent is generated only in the region where all three principal layers overlap (Fig. 1C). The origin of the photocurrent can be explained by examining the collective band diagram. In the idealized case, the structure is symmetric (Fig. 2B) and the electrons/holes generated in TMDC (by absorption of a photon with sufficient energy) have no preferred diffusion direction and, hence, no net photocurrent is measured. However, in the presence of a built-in electric field (Fig. 2C) across the TMDC

[either due to a difference in the initial doping between the graphene sheets or by gating (9)], the e-h pairs are separated and a photocurrent measured.

Immediately after fabrication (which involves the annealing stage) in the undoped state, the devices showed a minimum in the integrated photocurrent close to zero gate voltage (V_g) (Fig. 1D). For any finite V_g (either positive or negative), the photocurrent increased proportionally to V_g but began to saturate at $\sim \pm 20$ V, again due to the fi-

nite number of generated charge carriers. We also intentionally doped the top graphene electrode in one of our nonencapsulated samples to become p-type by exposing it to high-concentration water vapor. The photocurrent (Fig. 1E) at zero gate voltage became finite (positive), and the response with gate voltage was shifted by ~ 20 V. The effect is also seen in Fig. 2A, where the intercept of the *I-V* curves is shifted due to movement of the chemical potential in graphene. Our devices also

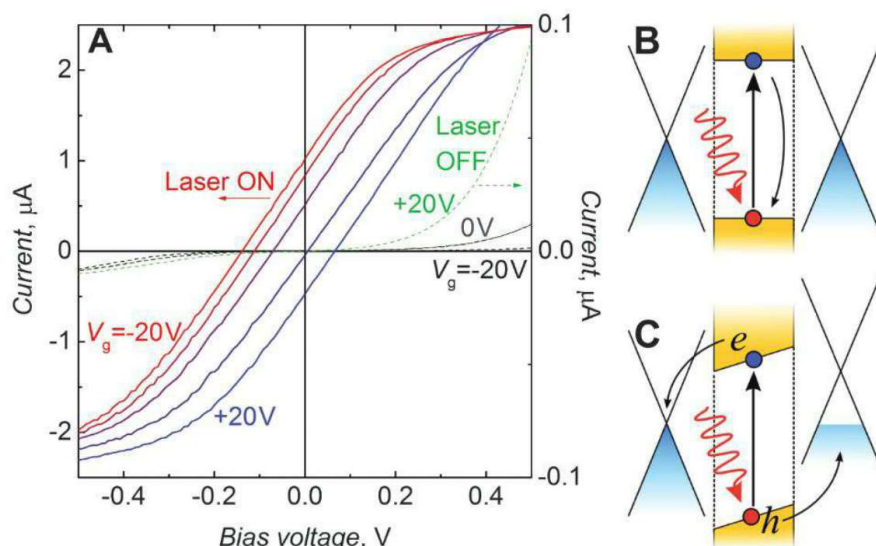
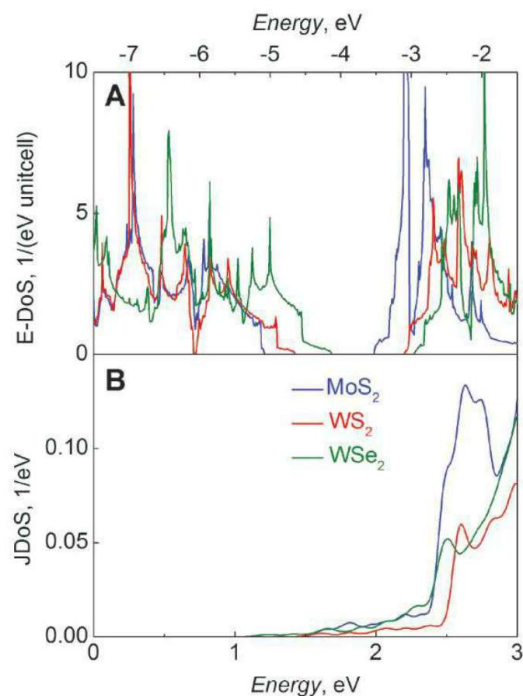


Fig. 2. Gate-dependent *I-V* characteristics. (A) (Left axis) *I-V* curves for a device on Si/SiO₂ taken under illumination at gate voltages from -20 (red) to $+20$ V (blue) in 10-V steps, after doping. The laser illumination energy was 2.54 eV and the power was 10 μ W. The curves are linear at low bias but saturate at higher bias due to limited available charge carriers. (Right axis) *I-V* curves for the same device taken in the dark at gate voltages from -20 (black) to $+20$ V (green) in 20-V steps, after doping. (B and C) Schematic band diagram for Gr/WS₂/Gr heterostructure with (C) and without (B) a built-in electric field to separate the generated e-h pairs.

Fig. 3. Electronic DoS for single-layer TMDCs. (A) The DoS for monolayer TMDCs: MoS₂, WS₂, and WSe₂. Strong peaks are present in all three materials that lead to a strong light-matter interaction. (B) The JDOS with the same three TMDC materials.



showed strong gate dependence without illumination, demonstrating transistor behavior. The on/off ratio (highest to lowest current modulation) of such tunneling transistors exceeds that of previously reported devices (9). Devices made from micromechanically cleaved and CVD graphene demonstrate very similar photovoltaic and transistor behavior, opening a way for scale-up.

The photocurrent observed in these devices is surprisingly strong for only a few atomic layers of TMDC, but this strong light-matter interaction can be understood from the nature of the electronic states in this material. *Ab initio* calculations (27) for the density of states (DoS) and the joint density of states (JDoS) of three single-layer semiconducting TMDCs (WS_2 , WSe_2 , and MoS_2) show strong peaks in the visible range (Fig. 3A) associated with Van Hove singularities in the DoS. This leads to enhanced light absorption and, importantly, this is a feature that is universal to TMDCs. These Van Hove singularities come from the nature of the electronic wave functions: Whereas the valence band is essentially composed of states coming from the d orbitals of the transition metal (TM), the conduction band is characterized by a linear superposition of d orbitals of the TM and p orbitals of the chalcogen atoms. The d orbitals have a localized nature with enhanced interaction effects. The p orbitals generate the σ bands, which in turn are responsible for the structural stability

of these materials [analogous to what happens in graphene (30)]. The localized character of the electronic bands (that is, the large effective mass of the carriers) leads to the peaks—i.e., Van Hove singularities—in the DoS, which are responsible for the enhanced photoresponsivity of these materials from the nanoscopic down to atomic scale. A direct measure of the effect of the Van Hove singularities in the optical response of TMDC is given by the JDoS, defined as

$$J\text{DOS}(E) = \frac{1}{4\pi^3} \int d^3k \delta(E_{V,k} - E_{C,k} - E)$$

where V and C are the valence and conduction bands, respectively. The JDoS is a direct measure of the so-called joint critical points, that is, the Van Hove singularities in the Brillouin zone around which a photon of energy, $\hbar\omega = E_C - E_V$, is very effective in inducing electronic transitions over a relatively large region in momentum space. The large contribution to the transition probability for joint critical points gives rise to the structure observed in the frequency dependence of the optical properties of the TMDC. Thus, the photocurrent, $I(\omega)$, at some light frequency ω is proportional to $J\text{DOS}(\hbar\omega)$ (31). There is a sharp rise in the photo-absorption in the $J\text{DOS}(E)$ in the visible range of all TMDCs studied (Fig. 3B). To further confirm that our results are not dependent on the thickness of the TMDC, we calculated the

DoS and JDoS for bulk (3D) semiconducting TMDCs (27). The peaks in the DoS and the sharp rise of the JDoS are comparable with the values found for a single layer in Fig. 3B and are consistent with the previous measurements on bulk MoS_2 (32). Hence, the strong light-matter interactions in semiconducting TMDCs are not a unique feature of the bulk material and can be extended to monolayers.

The effect discussed is similar, albeit with a different physical origin, to the strong Raman absorption in 1D semiconducting carbon nanotubes. In that case, the 1D nature of the material leads to $1/\sqrt{E}$ singularities in the DoS at the top (bottom) of the valence (conduction) bands, leading also to strong light-matter response (33).

We have also computed the work function, Φ , for the semiconducting TMDCs studied here. We find that the work functions vary considerably depending on the transition metal used (for monolayer, $\Phi_{\text{WS}_2} \sim 4.6$ eV, $\Phi_{\text{WSe}_2} \sim 4.3$ eV, and $\Phi_{\text{MoS}_2} \sim 5.1$ eV) and their thickness (for bulk, $\Phi_{\text{WS}_2} \sim 4.2$ eV, $\Phi_{\text{WSe}_2} \sim 3.9$ eV, and $\Phi_{\text{MoS}_2} \sim 4.5$ eV). Notice that as the work-function of graphene is comparable in magnitude ($\Phi_G \sim 4.5$ eV), it has been shown (34) that it has a very minimal effect on the band structure of TMDC, and the Dirac point of graphene stays within the gap, facilitating efficient extraction of both electrons and holes from TMDC.

We investigated in detail the performance of our prototype photovoltaic devices. An important parameter is the extrinsic quantum efficiency (EQE), defined as the ratio of the number of charge carriers generated to the number of incident photons. This can be expressed in terms of the photocurrent I , incident power per unit area P , and excitation wavelength λ by

$$\text{EQE} = \frac{hc}{e} \frac{I}{P\lambda}$$

where h is the Planck constant, c the speed of light in vacuum, and e the electron charge. Using the relation for EQE, we calculate the efficiency (Fig. 4), where the data were collected for several wavelengths at zero bias and $V_g = -40$ V. The extrinsic quantum efficiency did not appear to be dependent on wavelength, as expected from the approximately constant optical absorption, over this range (21). It is likely that the decrease in quantum efficiency with increasing power is due to screening of the built-in electric field by the excited electrons in the conduction band of WS_2 .

The already good performance and high EQE of our devices (ensured by the peculiar band structure of TMDC used) can be further improved by optimizing light absorption in the active layer. One possible way—the use of optical resonators (35)—is already partly realized in our devices on SiO_2 , where light interference in SiO_2 layer (36, 37) enhances the optical electric field in TMDC (this is one of the reasons for better performance of our devices on SiO_2 in comparison with those on flexible substrates). Another strategy is the use of plasmonic nanostructures (38–40) or metamaterials (41). To test the idea, we applied

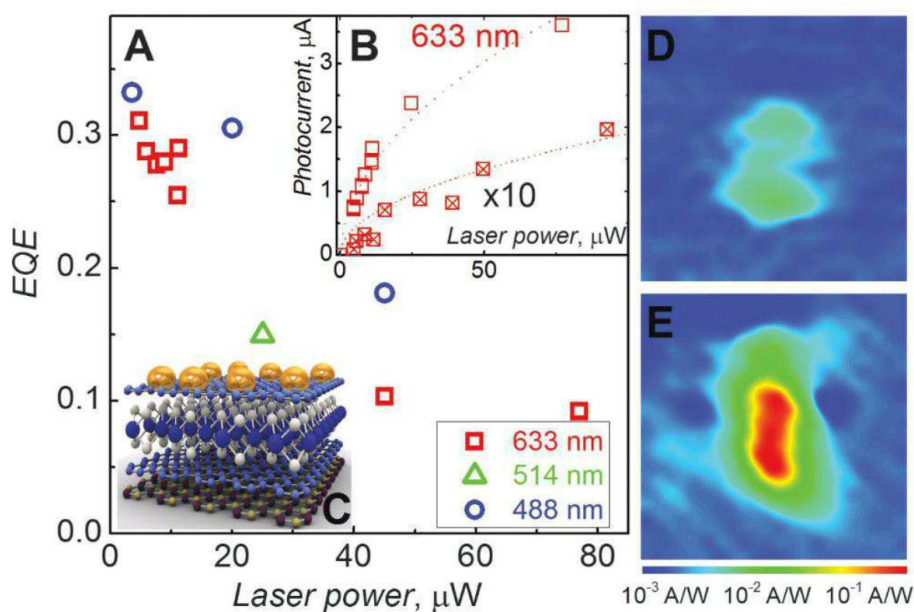


Fig. 4. Quantum efficiency. (A) The external quantum efficiency of the devices is the ratio of the number of measured e-h pairs to the number of incident photons. Due to the small variation in optical absorption across this wavelength range, the data for different wavelengths collapse onto a single curve. (B) Photocurrent measured with a 1.95-eV laser as a function of intensity; notice the sublinear dependence with laser power. This results in the largest quantum efficiency values at low intensities. Open symbols are for a device on Si/SiO_2 substrate, and crossed symbols are for a device on a flexible substrate. (C) Schematic representation of hBN/Gr/MoS₂/Gr (layers bottom to top) photovoltaic device with gold nanoparticles sputtered on top of the top graphene layer for plasmonic enhancement of light absorption. (D and E) Photocurrent maps of one of our hBN/Gr/MoS₂/Gr devices taken before (D) and after (E) sputtering of gold nanoparticles for plasmonic enhancement [illumination parameters: 633 nm, 10 μW; scan size, 14 μm by 14 μm; note the logarithmic scale chosen to represent the 10-fold increase in the photocurrent on (E)].

gold nanospheres (Fig. 4C) on top of one of our hBN/Gr/MoS₂/Gr heterostructures, which enhanced the optical field in the active layer and allowed for a 10-fold increase in the photocurrent, (Fig. 4, D and E) [see (27) for further details and other examples of the use of plasmonic nanostructures].

Atomically thin heterostructures of semiconducting TMDC present strong light-matter interactions that can lead to large photon absorption and photocurrent production. We are able to reach an extrinsic quantum efficiency of 30%, due to the localized character of the electronic wave functions in TMDCs that leads to large peaks in the DoS associated with van Hove singularities. The same devices demonstrate transistor behavior with on/off ratios exceeding those in previously reported devices. The use of various TMDCs, as well as their combinations, would allow one to create new transparent and flexible photonic and optoelectronic structures and devices with unique properties that surpass current technologies.

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Supplementary Materials

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Redox Heterogeneity in Mid-Ocean Ridge Basalts as a Function of Mantle Source

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The oxidation state of Earth's upper mantle both influences and records mantle evolution, but systematic fine-scale variations in upper mantle oxidation state have not previously been recognized in mantle-derived lavas from mid-ocean ridges. Through a global survey of mid-ocean ridge basalt glasses, we show that mantle oxidation state varies systematically as a function of mantle source composition. Negative correlations between Fe³⁺/ΣFe ratios and indices of mantle enrichment—such as ⁸⁷Sr/⁸⁶Sr, ²⁰⁸Pb/²⁰⁴Pb, Ba/La, and Nb/Zr ratios—reveal that enriched mantle is more reduced than depleted mantle. Because carbon may act to simultaneously reduce iron and generate melts that share geochemical traits with our reduced samples, we propose that carbon creates magmas at ridges that are reduced and enriched.

The composition and geophysical properties of Earth's mantle have evolved in response to oxygen fugacity (*f*O₂), a measure of the chemical potential of oxygen in solid systems (1, 2). Mantle-derived mid-ocean ridge basalts (MORBs) record *f*O₂ through the ratio of oxidized to total iron (Fe³⁺/ΣFe) (3),

and, because MORBs also record geochemically distinct mantle reservoirs, the potential exists to discover the existence and evolution of heterogeneities in the oxidation state of the mantle. Two previous large (*n* > 75) global surveys of Fe oxidation states in MORB pillow glass (4, 5) found no correlation between Fe³⁺/ΣFe ratios and mantle source composition, establishing the paradigm that oceanic upper mantle oxidation state is relatively uniform, buffered, and not linked to plate tectonic-scale processes. Other work (6) has proposed that enriched mantle domains may be more oxidized than normal MORB. We deter-

mined high-precision (±0.005) Fe³⁺/ΣFe ratios by micro-x-ray absorption near-edge structure (XANES) (7, 8) and trace element concentrations on 19 glasses (from seven geographical locations) that have not experienced substantial fractionation [i.e., primitive MORB with MgO > 8.5 weight % (wt %)] or plume influence (9) (table S1). Additionally, a partially overlapping set of 22 glasses (from 10 geographical locations) from ridge segments without plume influence, irrespective of MgO content, previously published Sr ± Pb ± Nd isotope ratios (table S1). The primitive data set spans 50% of the global range in Fe³⁺/ΣFe ratios, whereas the isotope data set spans the entire global range (fig. S1). Globally, the Fe³⁺/ΣFe ratio in MORB negatively correlates with MgO concentration, whereby the Fe³⁺/ΣFe ratio increases by ~0.03 as MgO decreases from 10 to 5 wt % (8) because Fe²⁺ preferentially partitions into fractionating mafic phases. In order to account for the effect of fractionation, the Fe³⁺/ΣFe ratios have been recalculated to an arbitrary reference value at MgO = 10 wt %, Fe³⁺/ΣFe₍₁₀₎, analogous to Fe₂O₃₍₈₎ in (4, 8). This correction is ~2% relative for the 19 primitive samples and is up to 11% (average of 7%) relative for the samples with isotopic data, but correlations between Fe³⁺/ΣFe ratios, trace elements, and isotopes are also evident in the uncorrected data (9) (fig. S1).

The glasses form subparallel arrays in ²⁰⁸Pb/²⁰⁴Pb–²⁰⁶Pb/²⁰⁴Pb space as a function of Fe³⁺/ΣFe ratio, with ²⁰⁸Pb/²⁰⁴Pb ratios increasing as

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a strong function of decreasing Fe oxidation state (Fig. 1, A and E). Reduced glasses also possess elevated $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and tend to have lower $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (Fig. 1, B and F). Oxygen fugacity, calculated from $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratio and glass composition (10) and referenced to the quartz-fayalite-magnetite buffer (ΔQFM), also correlates with isotopic enrichment (Fig. 1, C and D).

These observations link the Fe oxidation state of erupted MORB to mantle source heterogeneity, with enriched samples more reduced than depleted ones, because no magmatic process can fractionate these isotopes. Moreover, because these signatures require ancient fractionation of radiogenic parent-daughter pairs, these data also require preservation of the factors that lead to

heterogeneity in $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratio on plate tectonic time scales. For the primitive samples, $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratios correlate strongly with enrichment in highly incompatible elements (e.g., Ba, Th, and Nb) (Fig. 2) such that the most enriched samples are also the most reduced. Moderate correlations are also evident between $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratio and depletion of the high field strength elements Hf and Zr (Fig. 2, E and F). We define a Hf anomaly, Hf/Hf^* , relative to elements with similar compatibility during mantle melting, such as Sm and Nd [$\text{Hf}/\text{Hf}^* = \text{Hf}_N / \sqrt{(\text{Sm}_N \times \text{Nd}_N)}$], and observe that reduced samples also tend to have more negative Hf anomalies (fig. S3). By contrast, oxidation state does not correlate with ratios of mid to heavy rare earth elements such as Sm/Yb or Dy/Yb; the heavy rare earth element patterns in these samples are flat (fig. S4).

These data require a process that links source enrichment to a reduced oxidation state. This is contrary to the relationship expected if redox heterogeneities simply reflect a difference in the solid/liquid partition coefficient (D) between the two Fe species (i.e., $D^{\text{Fe}^{3+}} < D^{\text{Fe}^{2+}}$) (4, 5) or if enriched MORB derived from graphite-buffered melting at greater depth (6, 11), both of which would predict MORB enriched in incompatible elements to be more oxidized. Garnet-bearing lithologies previously implicated in the generation of enriched MORB [e.g., (12)] might hold back Fe^{3+} during melting; however, a silicate melt of a garnet-bearing source is inconsistent with the trace and major element characteristics of our reduced samples (supplementary text and figs. S4 and S5). Here, we hypothesize that control over the Fe oxidation state of MORB is exercised by another incompatible element: carbon.

Carbon concentration has the potential to control the eruptive $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios of MORBs such that the most-reduced basalts derive from sources with greater carbon concentrations (13). This is because reduced carbon, stabilized at depth by lower f_{O_2} (2), must fully oxidize upon ascent to be consistent with the oxidation state of the erupted basalts (8). Ferric iron becomes reduced in the process in proportion to the initial carbon content (13, 14). To generate the observed range of $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratios in either the primitive or isotope data sets solely through reduction of Fe_2O_3 by carbon requires variations in mantle C on the order of 80 to 170 parts per million (ppm). Independent estimates of mantle C concentration, which covary with enrichment (15), range from ~16 (depleted mantle) to >300 (enriched mantle) ppm (16). Thus, carbon may exert a primary influence on MORB oxidation state even if the erupted melts are too oxidized to be in equilibrium with graphite (8).

We cannot directly assess whether carbon concentration and oxidation state are correlated in our samples because CO_2 is partially degassed from most, if not all, MORB (17). Mantle carbon is constrained in two locations, however, and we note that trace element and carbon-enriched sample 2 π D43 (popping rock) suggests a mantle

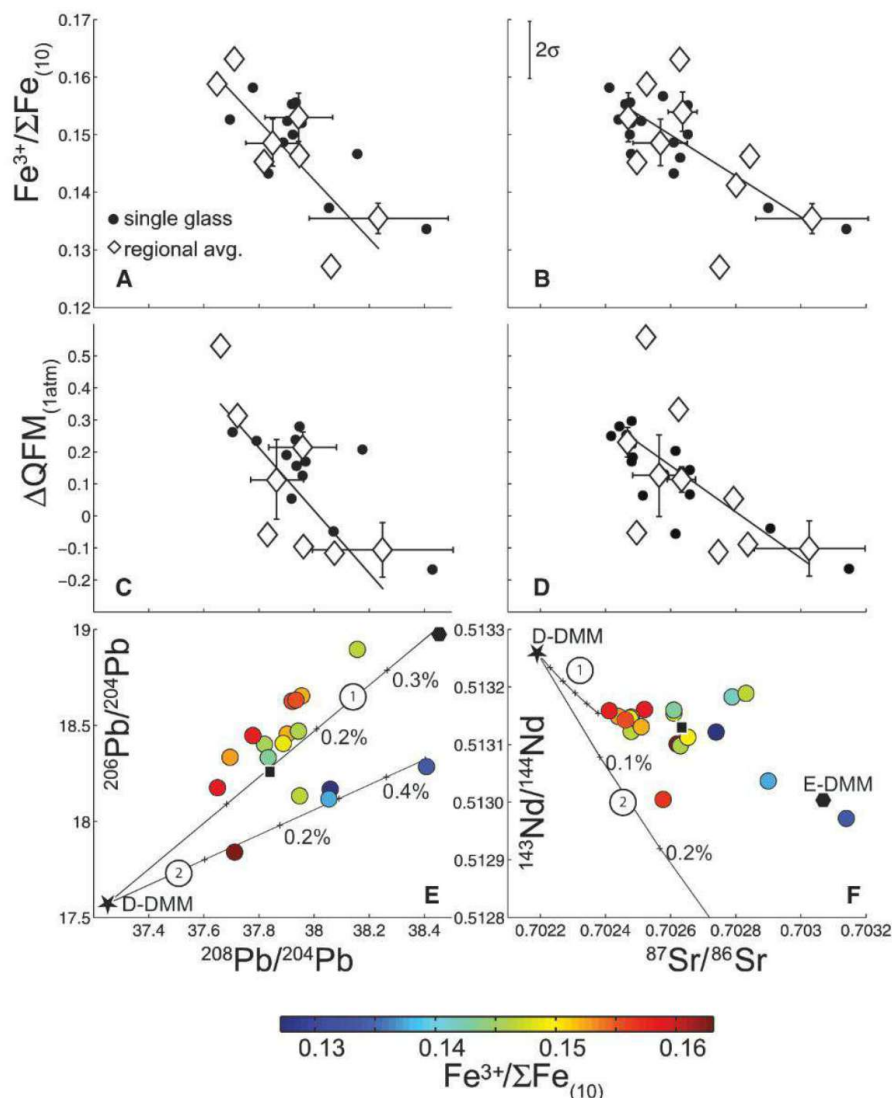


Fig. 1. Decrease in $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratios as a function of isotopic enrichment. MORB $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios versus $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratios (A and B) and f_{O_2} relative to the QFM buffer at 1 atm (C and D). $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratio accounts for 50 and 44% of the variance in these isotopic ratios, which is statistically significant at $P \leq 0.001$ (F -test results, table S3). Solid circles show individual analyses, and open diamonds show the regional averages and $\pm 1\sigma$ variability for each geographic location. MORB $^{208}\text{Pb}/^{204}\text{Pb}$ versus $^{206}\text{Pb}/^{204}\text{Pb}$ ratios (E) and $^{87}\text{Sr}/^{86}\text{Sr}$ versus $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (F) as a function of $\text{Fe}^{3+}/\Sigma\text{Fe}_{(10)}$ ratios, showing a decrease in the oxidation state of Fe in the glasses as a function of isotopic enrichment. Color bar shows the relative Fe oxidation state of each sample. Curve 1 models 0.1% additions of a low-degree carbonatitic melt of subducted material to depleted-depleted MORB mantle (D-MMM; star), generated 2 Ga after subduction. Depleted (square) and enriched (hexagon) MORB mantle are from (20). Curve 2 demarcates additions of a low-degree carbonatitic melt of the same subducted source material as 1 but with the carbonatitic melt generated immediately after subduction at 2.8 Ga. The difference between these two curves is timing of the parent-daughter fractionation introduced by carbonatitic melting, where curve 1 assumes no fractionation of the subducted material until melting beneath the mid-ocean ridge and curve two assumes carbonatitic melt-induced fractionation immediately after subduction. Errors in isotopic ratios are as provided by the authors of those studies (9).

source with ~159 ppm CO₂ (18) and is more than two standard deviations more reduced than the global mean, whereas the trace element–depleted Siqueiros fracture zone basalts indicate ~72 ppm CO₂ in the source (19) and are among the most oxidized in our suite (Fig. 2 and fig. S1). Critically, some of the geochemical signatures most highly correlated with oxidation state [e.g., isotopes tending toward the EM-1 (enriched mantle) end member (20); elevated Ba/La, Th/La, Nd/Hf, Ba/Rb, Nb/Ta, and Nb/La ratios; and negative Hf anomalies] are not easily generated by silicate melting but are a natural consequence of melting in the presence of carbon (supple-

mentary text and fig. S3). Low-degree carbonatitic or kimberlitic melts may extract the highly incompatible elements to the melt phase while leaving Hf and Zr in the residue (21–23). Carbonatitic melts also fractionate radiogenic parent-daughter pairs, such that carbonatitic melts evolve more-radiogenic Sr and Pb and less-radiogenic Nd isotopic ratios over time, toward the EM-1 mantle component (22). Our samples are geographically distributed (fig. S2) and are not genetically related. Thus, it is not sensible to develop a petrogenetic model that accounts for each sample's full major, trace, and radiogenic element signature. However, we do

show in Figs. 1 and 2 that addition of a few tenths of a percent of low-degree carbonatitic melt of subducted material to depleted silicate melt generates trace element and isotopic arrays that reproduce the most salient geochemical signatures associated with low Fe³⁺/ΣFe ratios (supplementary text).

The deep Earth is a large reservoir for carbon, continually replenished by subduction (24, 25), and thus the mixtures of low-degree carbonatitic and/or kimberlitic melts and high-degree silicate melts may be widespread depending on the distribution of carbon in the deep Earth (13, 23, 26). Before 2.3 billion years ago (Ga), anoxic conditions at Earth's surface (27) would have resulted in subduction of reduced carbon associated with trace- and isotopically enriched sediment and crust into mantle that was already relatively oxidized (28). Subduction at 2.8 Ga may have created reduced domains in the mantle while enabling carbonate-fluxed melting to fractionate parent-daughter pairs consistent with those observed (Fig. 1). Today, the mantle's descending *f*_{O2} gradient should immobilize carbon through redox freezing (29); however, the potential of subducted carbonate to generate mobile reduced carbon species (2) cannot be ruled out. Any mobilization of reduced carbon may, upon decompression, result in melts that are simultaneously enriched and reduced (supplementary text and fig. S6). Additional mechanisms may exist to create geochemically enriched reducing domains in the mantle (30), but their geochemical implications for MORB are still unknown.

Our observations have important implications for the persistence of heterogeneities in mantle oxidation state through time. Far from being homogeneous or well-buffered, the mantle appears capable of retaining oxidation state information over plate tectonic time scales. This implies that redox-active elements such as H, C, S, and Fe do not buffer the upper mantle at uniform *f*_{O2}. Rather, the Fe³⁺/ΣFe ratios of MORBs, like arcs (31), reflect variations in their sources.

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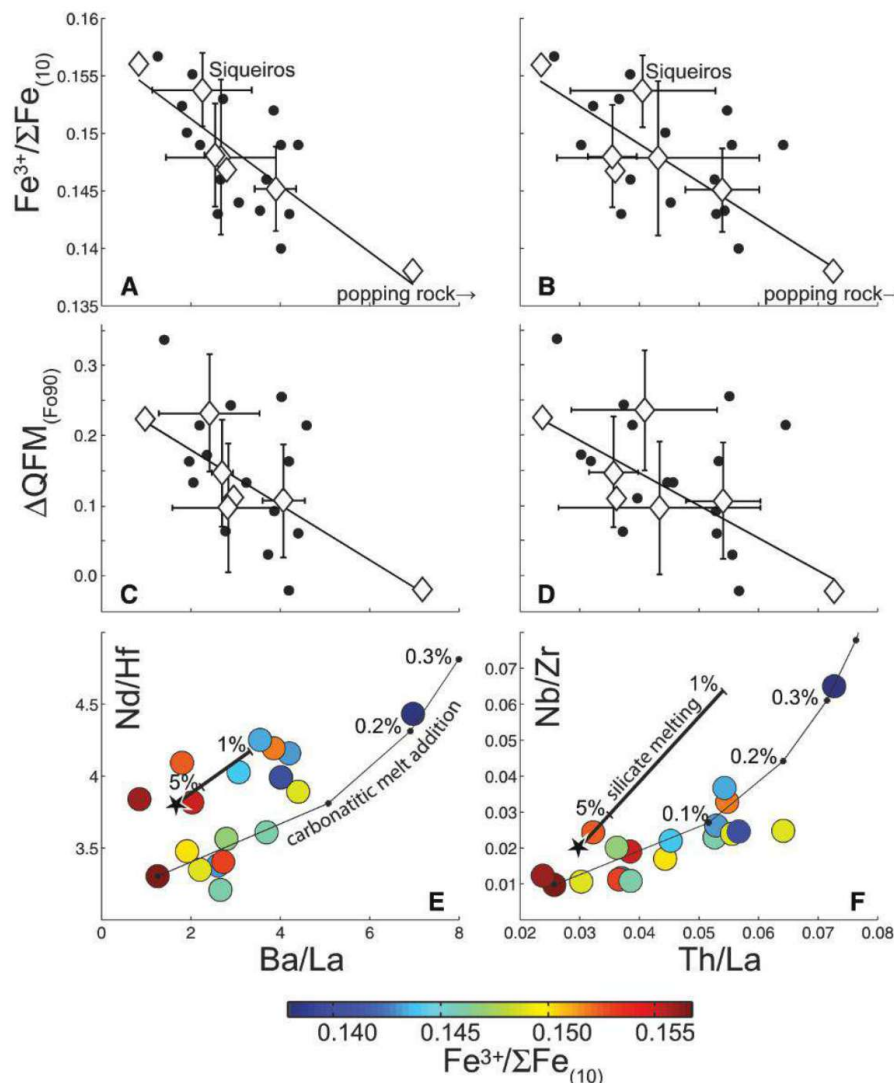


Fig. 2. Decrease in Fe³⁺/ΣFe₍₁₀₎ ratios with trace element enrichment in primitive glasses with >8.5 wt % MgO. (A and B) Ba/La and Th/La ratios decreasing as a function of Fe³⁺/ΣFe₍₁₀₎ ratio and the *f*_{O2} of the source mantle at the average pressure and temperature of melt segregation (~0.7 to 1.3 GPa) relative to the QFM buffer (8) (C and D). Fe³⁺/ΣFe₍₁₀₎ ratio accounts for 53 and 43% of the variance in these trace element ratios, statistically significant at *P* ≤ 0.002 (*F*-test results, table S3). Solid circles show individual analyses, and open diamonds show the regional averages and ±1σ variability for each geographic location (popping rock Ba/La = 13.2, Th/La = 0.11, Fe³⁺/ΣFe₍₁₀₎ = 0.137). (E and F) Covariation of Ba/La with Nd/Hf ratios and Th/La with Nb/Zr ratios as a function of Fe³⁺/ΣFe₍₁₀₎ ratio. Lines model 0.1% additions (+) of a low-degree carbonatitic melt of subducted material to depleted sample VG5211 from Siqueiros (supplementary text). D-DMM (star) shown with silicate melt trajectory to 1% melt fraction. Errors in trace element ratios are smaller than the symbol sizes.

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Supplementary Materials

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Hydrogen Isotopes in Lunar Volcanic Glasses and Melt Inclusions Reveal a Carbonaceous Chondrite Heritage

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Water is perhaps the most important molecule in the solar system, and determining its origin and distribution in planetary interiors has important implications for understanding the evolution of planetary bodies. Here we report in situ measurements of the isotopic composition of hydrogen dissolved in primitive volcanic glass and olivine-hosted melt inclusions recovered from the Moon by the Apollo 15 and 17 missions. After consideration of cosmic-ray spallation and degassing processes, our results demonstrate that lunar magmatic water has an isotopic composition that is indistinguishable from that of the bulk water in carbonaceous chondrites and similar to that of terrestrial water, implying a common origin for the water contained in the interiors of Earth and the Moon.

During the solar system's early stages, the solar nebula was cold enough to allow the condensation of water into ice only beyond a distance of ~1 to 5 astronomical units (AU), termed the snowline (where 1 AU is the Earth-Sun distance). Planetesimals accreted beyond this distance grew into water-rich bodies, whereas those accreted closer to the Sun were devoid of water, resulting in relatively H₂O-depleted terrestrial planets and H₂O-enriched giant planets (1). Dynamical models suggest that water and other volatiles in the terrestrial planets have been the result of the accretion of volatile-rich asteroids coming from two different source regions at two distinct times: one from inside the Jupiter-formation region and the other between and beyond the giant planets, 5 to 100 million years (My) and >300 to 500 My after the formation of the first solids, respectively (1, 2).

Hydrogen isotopes (deuterium, D; and hydrogen, H) provide unique insight into the origin of water in planetary bodies (3–7). The solar sys-

tem consists of reservoirs containing water with an extremely wide range of D/H ratios (8). The variation in D/H ratios partly reflects the primordial gradient of water and other volatiles through the solar system as a function of distance from the

Sun (Fig. 1). Deuterium depletion is characteristic of the protosolar nebula (7), whereas the water ice from the outer solar system, such as in the Oort cloud comets, is enriched in D/H ratios by a factor of 2 or more over terrestrial water (3–7). The bulk water in carbonaceous chondrite meteorites (3) has a D/H ratio similar to that of Earth's water (9), suggesting that these meteorites might be responsible for bringing water to the terrestrial planets. However, a terrestrial-like D/H ratio has recently been measured on a Jupiter-Family comet (JFC), raising the possibility that Earth's water could have originated from cometary material (10). The similar D/H ratios among CI-CM carbonaceous chondrite meteorites, JFCs, and Earth may support the hypothesis of a common source region for the water of these celestial objects (3, 10, 11).

The Moon is thought to have formed in a giant collision between a planet and an early formed proto-Earth (12, 13). Although it has long been considered that this event removed essentially all hydrogen from the Moon (14), recent measurements on lunar volcanic glasses, melt inclusions, lunar apatites [Ca₅(PO₄)₃(F,Cl,OH)], and

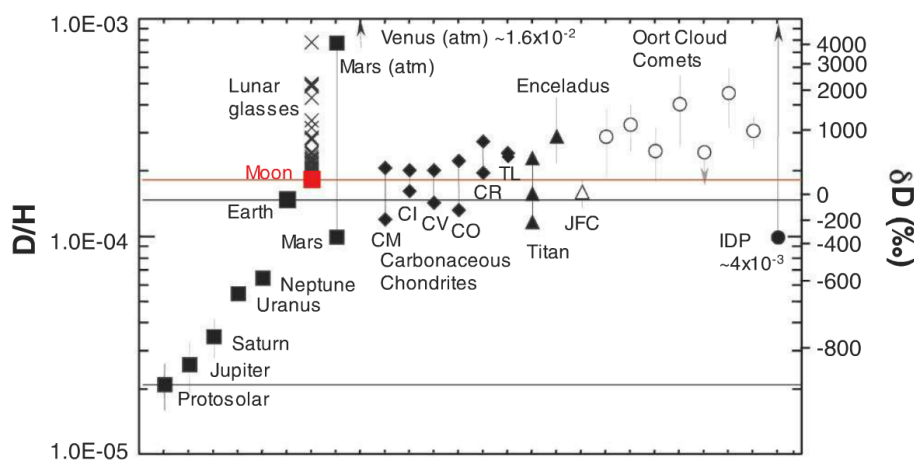


Fig. 1. The range of hydrogen isotopic composition in solar system objects. X symbols are the δD values of individual glass beads and melt inclusions corrected for cosmogenic contributions of H and D (see table S1). The glasses with errors $\pm 1000\%$ after spallation correction were not considered. The value for the lunar mantle (red square) represents the lowest δD and the highest water content measured in lunar melt inclusions. IDP, interplanetary dust particles; JFC, Jupiter-family comet. See (21) for references.

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plagioclase from ferroan anorthosites have demonstrated that the Moon's interior is not devoid of water (15–20). To the contrary, some of these reports showed that volatile depletion in the lunar lavas is the result of magmatic degassing, and that some of the lunar melts originally contained as much water as terrestrial mid-ocean ridge magmas (15, 19).

Here we present the isotopic composition of hydrogen dissolved in lunar volcanic glasses and in their olivine-hosted melt inclusions to establish the source of the lunar magmatic water. These volcanic glasses, returned by the Apollo 15 and 17 missions, represent some of the best-studied and most primitive magmas generated within the Moon (14, 21). We measured the D/H ratios and H₂O contents simultaneously in the center of the exposed interiors of individual lunar volcanic glass beads and olivine-hosted melt inclusions using a Cameca NanoSIMS 50L multicollector ion microprobe (21). We examined very-low-Ti and low-Ti glasses from Apollo 15 15426/27 and high-Ti glasses from Apollo 17 74220. The Apollo 17 high-Ti glasses contain olivine-hosted melt inclusions, small samples of magma trapped within the olivine that grew in the magma before eruption. By virtue of their enclosure within their host crystals, melt inclusions are protected from loss of volatiles by degassing during eruption. Thus, melt inclusions have the highest concentrations of water, up to 1200 parts per million (ppm) (19). The matrix glasses surrounding the olivines with melt inclusions contain 6 to 32 ppm H₂O, and other Apollo 17 high-Ti glasses contain 3 to 9 ppm water. Apollo 15 low-Ti and very-low-Ti glasses range from 16 to 69 ppm and 4 to 34 ppm water, respectively. All of the glass beads and melt inclusions are enriched in deuterium compared with terrestrial ocean water, with δD values ranging from +189 per mil (‰) up to +5023‰ (table S1).

Care must be taken when interpreting variations in the D/H ratios of planetary volcanic rocks in order to distinguish process-related D/H variations from source-related D/H composition. Thus, before the D/H ratio can be used as a source indicator for planetary water, it is important to consider secondary processes that can change this ratio (21). Our data exhibit a negative correlation of D/H ratio with water content (fig. S1), a feature of the entire data set independent of the compositional type of glass measured. This correlation points to a set of processes that have modified the original magmatic water present in these lunar magmas during and/or after their eruption. Solar wind implantation, cosmic-ray spallation, and magmatic degassing can all potentially modify the water content and D/H ratios. Although the effect of solar wind implantation is negligible in the samples studied here (21), cosmic-ray spallation and degassing processes have significantly affected the water and D/H ratios of the lunar glasses. However, as demonstrated in the following sections, these processes are inferred to have had minimal effect on the D/H ratio for the melt inclusions with high water content (21). The

melt inclusions therefore provide the most direct constraints on the isotopic composition of juvenile lunar water.

We calculate the cosmogenic contributions of D and H using the known H and D production rates (22, 23) and the average cosmic-ray exposure ages 284 ± 51 My and 30 ± 10 My for Apollo 15 and 17 glasses, respectively (24, 25). Once corrected for cosmogenic production (21), the total variation in D/H ratios among the lunar glasses is greatly reduced (Fig. 2). However, the

data, excluding glasses with water ≤ 10 ppm because of their large associated uncertainties, still define a slightly negative correlation of D/H ratio with water content, suggesting that degassing has also affected the isotopic composition (Fig. 3 and fig. S2).

It has been demonstrated that single lunar glass beads have core-rim diffusion profiles for H₂O and other volatiles, which is evidence for diffusion-limited kinetic degassing in a low-pressure environment after fragmentation of the magma

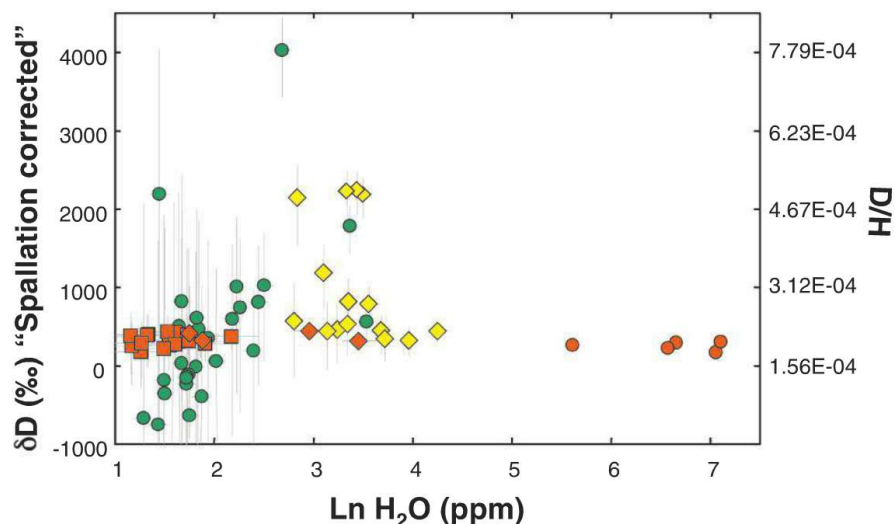


Fig. 2. Water contents and hydrogen isotope compositions of lunar volcanic glasses and melt inclusions corrected for spallation processes. Green circles: very-low-Ti glasses; yellow diamond: low-Ti glasses; orange circles, diamonds, and squares: high-Ti melt inclusions, matrix glass, and glass beads, respectively. The error bars represent propagation of errors in the analyses, exposure ages, and D and H production rates. See fig. S1 for measured values before correction (21).

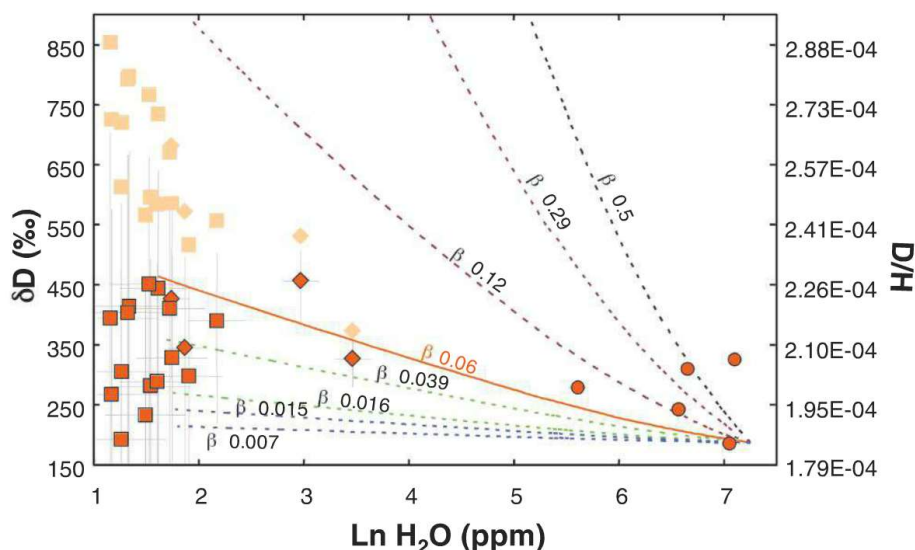


Fig. 3. Water contents and hydrogen isotope compositions of high-Ti glasses and melt inclusions. Errors and symbols as in Fig. 2. Light orange points represent measured data uncorrected for spallation. Curves represent 1D diffusive degassing model; full line encompasses the data with an empirical $\beta \leq 0.06$. Colored dashed lines indicate diffusive degassing of H₂, H₂O, and OH[−] with their values of β . See fig. S2 for comparison of all the data with degassing model; see also text and (21) for details.

during eruption (15). Therefore, it is important to consider how kinetic degassing might affect the D/H ratios of the magmatic water contained in lunar volcanic glasses. The mass dependence of diffusion of hydrogen isotopes can be represented as

$$\frac{D_H}{D_D} = \left(\frac{m_D}{m_H} \right)^{\beta_{H/D}} \quad (1)$$

where D is the diffusion coefficient, m is the atomic mass, and β is an empirical exponent that likely depends on hydrogen speciation (21). Under the reducing conditions relevant to lunar magmas, hydrogen may exist in several different molecular species— H_2 , H_2O , and OH^- —in proportions that depend on the total concentration of H, oxygen fugacity (f_{O_2}), pressure, and temperature (26, 27). The diffusivities of these species in silicate liquids and glasses are very different, with $D_{H_2} > D_{H_2O} \gg D_{OH^-}$ (26). The mass dependence of hydrogen isotope diffusion is also expected to be different for each of these species because of their different molecular masses and their different mechanisms of diffusive transport, with β values that could range from as high as 0.5 to as low as ~ 0.01 (21). From comparison of the D/H ratios and water contents of Apollo 17 high-Ti glasses and melt inclusions to the results of a simple one-dimensional (1D) spherical diffusive degassing model (Fig. 3), we obtain an empirical β that depends on the initial and final δD considered, but is constrained to be ≤ 0.06 . This β value is intermediate between those estimated for diffusion of OH^- and H_2 (21), the hydrogen species expected at the conditions of the lunar magmas (27). In the simple model presented here, H diffusivity and β are assumed to be constant during degassing. It is instead likely that β changed with progressive degassing, from initial values of 0.29

to 0.12, representing H_2 diffusion, to values as low as 0.015 to 0.007 in the later stages of degassing, where diffusion is expected to be predominantly as OH^- (21). A more complex modeling of the data considering these changes is unwarranted, however, given the large uncertainties in D/H ratios of glasses with water content lower than 10 ppm and the lack of experimental data on the diffusivities of the relevant hydrogen species in melts at variable hydrogen concentration, temperature, and f_{O_2} .

The primitive major-element compositions, the high water contents, and the minimal effect of spallation and degassing on the D/H ratios of Apollo 17 olivine-hosted melt inclusions indicate that they are ideal samples to determine the primitive δD of the lunar juvenile water. Melt inclusions provide an advantage over erupted lunar glasses because they are commonly trapped at pressures exceeding the pressure of eruption, and they are protected from eruptive degassing by their host crystal (28). However, there is no way of knowing how extensively lunar magmas have degassed before the melt inclusions formed. It is likely that these magmas underwent some degree of degassing before or during the formation of the melt inclusions. The presence of metal blebs trapped in olivine phenocrysts of Apollo 17 high-Ti glasses have been interpreted as evidence for magmatic degassing before olivine crystallization and melt inclusion formation (29). Therefore, it is expected that the original preeruptive δD value of these lunar magmas was lower, and that kinetic D/H fractionation has resulted in preferential loss of H during magmatic degassing before entrapment (28). Hence, our melt inclusion data (1200 ppm H_2O and $\delta D +187\%$ for the less degassed inclusion) indicate a lower limit on the water content and an upper limit on the primary δD of these

magmas. This δD value is similar to that measured in apatite ($+238\%$) from a 3.9-billion-year-old alkali anorthosite adcumulate, 14305 (18). If this cumulate crystallized as a closed system, then its δD may be comparable to the primitive D/H ratio of a reservoir in the lunar interior (21), supporting our assertion that the δD of the melt inclusions represent most closely the isotopic composition of the primitive lunar magmatic water.

The δD of lunar juvenile water ($\sim +187\%$) is within the range of carbonaceous chondrites (3), slightly heavier than that estimated for bulk Earth ($\sim -43\%$) (6), significantly lighter (by a factor of ~ 2) than those of the Oort cloud comets (5), but similar to the δD measured in the JFCs (10). However, when we combine our D/H results with the previously reported $^{15}N/^{14}N$ ratios for the Moon (30), the values for the lunar indigenous volatiles are within the range measured for carbonaceous chondrites, but quite distinct from those in comets, greatly limiting the possible cometary contribution to the lunar juvenile water and nitrogen budgets (Fig. 4). The lunar N and H isotopic compositions suggest that the volatile elements in the Moon originated from the same reservoir as the parent bodies of primitive meteorites, contrary to previous interpretation of δD of lunar apatites (18). Our results on lunar volatiles are consistent with a recent evaluation of the origin of Earth's volatile budget (3–5) and suggest a common origin for the water in Earth and Moon.

The similarity of both preeruptive H_2O content (19) and magmatic D/H ratios (this study) of the lunar glasses to terrestrial magmas supports the notion that the water from Earth and the Moon have a common origin. The Moon must have received its water during or shortly after its accretion, before the formation of a robust lunar lithosphere ≤ 100 My after the generation of the satellite. Data for highly siderophile elements (31) suggest that a late veneer of meteoritic material delivered to the Moon was too small to be responsible for the lunar volatile budget (15–20). Moreover, no quantitative model has been presented yet that convincingly explains the volatile and siderophile elements budget and isotopic composition for Earth and Moon by a late veneer (4, 5). Therefore, the simplest scenario consistent with our observations is that Earth was wet at the time of the Moon-forming impact, as predicted by dynamic models (1, 2), and that the water was not completely lost during this event. Furthermore, our results provide evidence that Earth's water budget and isotopic composition at the time of the giant impact were broadly similar to what they are today. The hydrogen isotopic similarity suggests that chemical exchange of even the most volatile elements between the molten Earth and the proto-lunar disc could have been pervasive and extensive, even at the very high temperatures expected after a giant impact. This could have been aided by the presence of a high-temperature convective atmospheric envelope

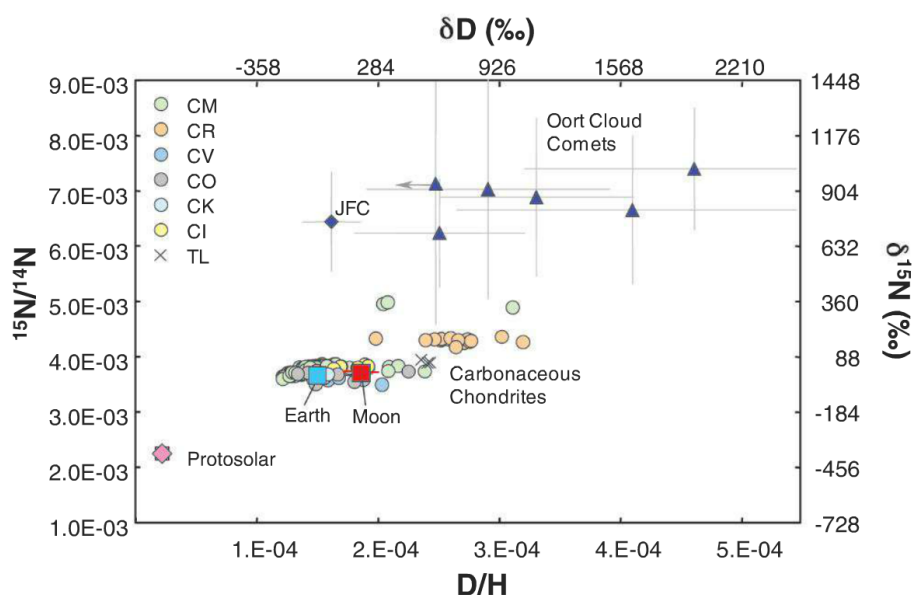


Fig. 4. D/H and $^{15}N/^{14}N$ ratios of the Moon compared to carbonaceous chondrites, Earth, and comets. The δD value for the lunar mantle (red square) represents the lowest value measured in lunar melt inclusions and the $\delta^{15}N$ from (30). Figure modified from (5). See (21) for details and references.

surrounding Earth and the proto-lunar disc as the Moon solidified (32). Alternatively, it is conceivable that a portion of the lunar interior escaped the widespread melting and degassing expected in the aftermath of a giant impact and simply inherited water from the proto-Earth. The latter alternative is consistent with the hypothesis that the Moon began with a 200- to 300-km-thick outer shell near melting conditions and a relatively cold interior (33). This hypothesis has received support from recent gravity gradiometry observations by the Gravity Recovery and Interior Laboratory (GRAIL) (34). Any dynamic model proposed for the formation of the Earth-Moon system must meet the constraints imposed by the presence of H₂O with an isotopic composition similar to that of terrestrial water in the lunar interior.

Our findings also have implications for the origin of water ice in permanently shadowed lunar craters, which has been attributed to solar wind implantation and to cometary and meteoritic impacts (21). It is conceivable that at least part of this water could have originated from magmatic degassing during lunar volcanic eruptions.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 and S2

Table S1

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Clarifying the Dominant Sources and Mechanisms of Cirrus Cloud Formation

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Formation of cirrus clouds depends on the availability of ice nuclei to begin condensation of atmospheric water vapor. Although it is known that only a small fraction of atmospheric aerosols are efficient ice nuclei, the critical ingredients that make those aerosols so effective have not been established. We have determined in situ the composition of the residual particles within cirrus crystals after the ice was sublimated. Our results demonstrate that mineral dust and metallic particles are the dominant source of residual particles, whereas sulfate and organic particles are underrepresented, and elemental carbon and biological materials are essentially absent. Further, composition analysis combined with relative humidity measurements suggests that heterogeneous freezing was the dominant formation mechanism of these clouds.

The effect of clouds on the climate system is more uncertain than the influence of heat-trapping greenhouse gases (1). Clouds can cool by reflection of solar radiation and warm by trapping terrestrial heat with the balance of effects depending on cloud properties such as altitude, thickness, phase, and droplet or crystal size (2). Cirrus clouds are of particular importance because they have extensive global coverage and occur high in the atmosphere, at altitudes of 8 to 17 km (2). Global modeling suggests that human effects on ice clouds may rival the radiative effect of all anthropogenic aerosol particles that do not participate in cloud formation (3).

Due to the temperature at their altitude of formation, cirrus clouds are composed exclusively of ice crystals (2). Ice nucleation does not take place directly from water vapor but instead requires a preexisting particle (4). Ice forms via two pathways, termed homogeneous and heterogeneous freezing. Homogeneous freezing, the spontaneous nucleation of ice within a sufficiently cooled solution, is better understood. A simple theoretical framework for this process has been developed for use in model studies (5). Because the vast majority of atmospheric aerosol particles are aqueous solutions of sulfates and organic molecules (6), homogeneous freezing has been as-

sumed the dominant process (7). However, one drawback to homogeneous freezing is that relative humidity must be strongly supersaturated with respect to ice ($RH_i = 150$ to 170%) (4, 8). In contrast, heterogeneous freezing can start just below 0°C and at $RH_i \sim 100\%$ (2, 8). Heterogeneous freezing remains poorly understood, however, because it can take several subpathways, among which are depositional freezing of water vapor onto a particle surface and immersion freezing from within an aqueous coating (4). Many materials have been shown to act as ice nuclei (IN) in laboratory experiments, including mineral dust, metallic particles, some biological materials, low-temperature glasses, and anhydrous salts (4, 9–11). Despite this variety, only a small fraction of atmospheric particles at ground level (as few as ~ 1 in 10^5) have been shown to act as IN (4, 8).

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Here, we have determined the chemical and physical properties of the particles on which cirrus ice crystals formed from measurements acquired aboard two NASA research aircraft. These

data reveal the particle types that are relevant for cirrus formation, from which we can infer the ice nucleation mechanism. We term the material within an ice crystal an ice residual (IR) because

particles and gases may be scavenged after ice nucleation. Our data do not indicate multiple particles per IR, and the low pressure and number density of particles in the cirrus regime render it unlikely that scavenging represents a distinct artifact (12).

Between 2002 and 2011, we conducted four aircraft measurement campaigns, designed to sample within regions of high cirrus cloud abundance, over North and Central America and nearby ocean (Fig. 1). Data from liquid water-containing clouds are excluded. Tropical tropopause cirrus clouds were previously considered (13). These clouds, which can have extensive coverage in the tropics, have been shown to consist of a low number density of relatively small ice crystals that probably formed heterogeneously on glassy aerosols or anhydrous salts (13). Measurements were taken in air-traffic corridors, although contrails were not specifically targeted. We then combined mass spectra for thousands of individual IRs and near-cloud aerosol (Fig. 2) into seven categories (Fig. 1). The mode of freezing is inferred from the relative composition: When the IRs were predominantly sulfate and/or organic and similar to the near-cloud particles, homogeneous freezing is inferred, whereas dissimilar IRs and near-cloud particles indicate heterogeneous nucleation (8, 14). Based on these criteria, the freezing mechanism was heterogeneous in 94% of cloud encounters.

The predominant particle category on which freezing took place was mineral dust and metallic particles, at 61% (Fig. 1). This category was also the most enhanced in cloud ice, with respect to its near-cloud abundance (5%). The overwhelming majority (~90%) of particles exhibit no apparent sulfate or organic coating. Mixtures of sulfate and organic carbon—in all cases, the largest near-cloud category at 55%—were the principal IRs in only two distinct cloud encounters for which homogeneous freezing is inferred. In all other cirrus clouds, sulfate and organic particles were 14% of the IRs. Sulfate and organic material can act as IN when present as glasses or anhydrous salts (10, 11). Therefore, some of these particles may have entered the ice phase heterogeneously, implying that the 94% value of heterogeneous freezing may be a lower limit. Sea salt was abundant as IR (at 25%) during flights that took place over open ocean but was only 3% IR during other flights. In all cases, biomass burning was the second most near-cloud-abundant particle type (36%) but was depleted in the IR, representing <5%. These quantities represent composite averages for each of the four campaigns. We individually analyzed single flights within campaigns, with the only differences from the campaign averages being the homogeneous freezing cases (12). Representative spectra are shown for four IR types in Fig. 2.

The cirrus encounters presented here include both convective outflow and synoptically formed clouds. Because of the updrafts in convection, the aerosols immediately next to these clouds may not be representative of those within. Analyzed separately, we find that mineral dust and metallic

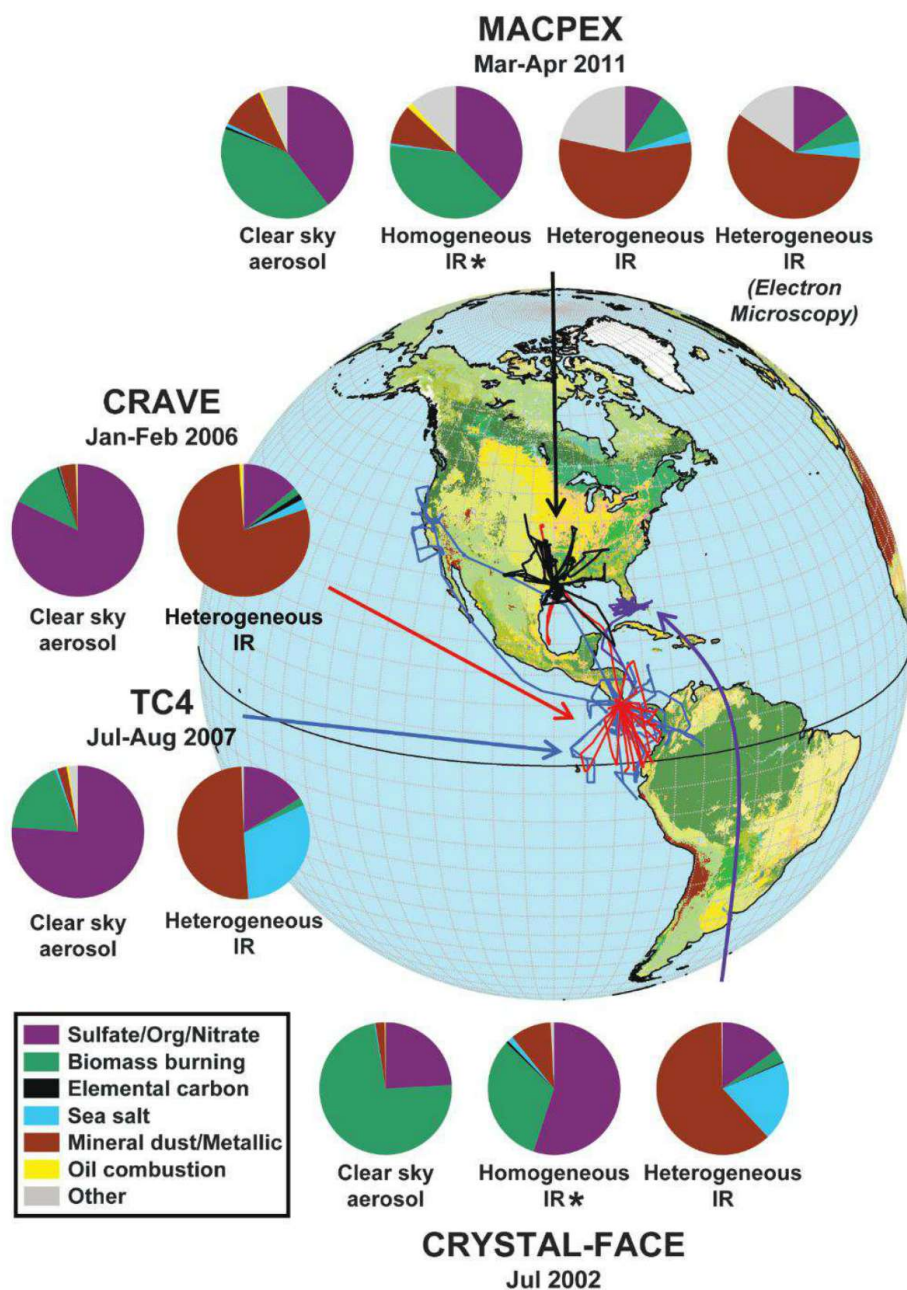


Fig. 1. Flight tracks of ice cloud-residual measurements for four aircraft campaigns spanning a range of geographic regions and seasons. The composition of cirrus IRs and near-cloud aerosol, on a number basis, are summarized for each campaign. Ice-crystal separation was accomplished with a counterflow virtual impactor inlet, and composition was determined by single-particle mass spectrometry (MS) where individual particles were desorbed and ionized with a 193-nm wavelength excimer laser and ion abundance was measured with time-of-flight MS (13, 14). This technique is sensitive to all atmospheric aerosol particle components in a range from ~0.2- to 3- μ m aerodynamic diameter (13, 14). IR particles were also collected and analyzed with EM coupled to energy-dispersive x-ray microanalysis (21). Homogeneous freezing appeared to initiate cloud formation for only two individual cirrus clouds with composition shown in two insets (denoted by asterisks). Mineral dust is the most dominant heterogeneous ice nucleus in all other cirrus encounters despite the geographic separation between the study areas and major global dust sources, shown in brown (NASA Land Processes Distributed Active Archive Center, https://lpdaac.usgs.gov/get_data).

particles are the dominant IRs, regardless of cirrus type, when heterogeneous freezing was inferred (56% in clouds associated with convection and 63% in those without). We therefore believe this result is consistent across cirrus-development scenarios. Individual field study values are provided in Fig. 1 (12).

Relative humidity measurements can also be used to constrain cirrus-formation mechanisms (15, 16). Clear-sky (cloud-free) RH_i measurements taken during the flights on which IR data were collected are shown as a function of temperature in Fig. 3, along with measurements from three campaigns spanning a larger geographic area

(17, 18). Sampling locations include North and Central America, the western Atlantic, and an area spanning from the central Pacific to the Arctic. At cirrus temperatures, $<3\%$ of the clear-sky RH_i data exceed $\sim 140\%$, a humidity consistent with heterogeneous freezing of mineral dust (2, 4). Less than 0.5% of the RH_i data surpass the

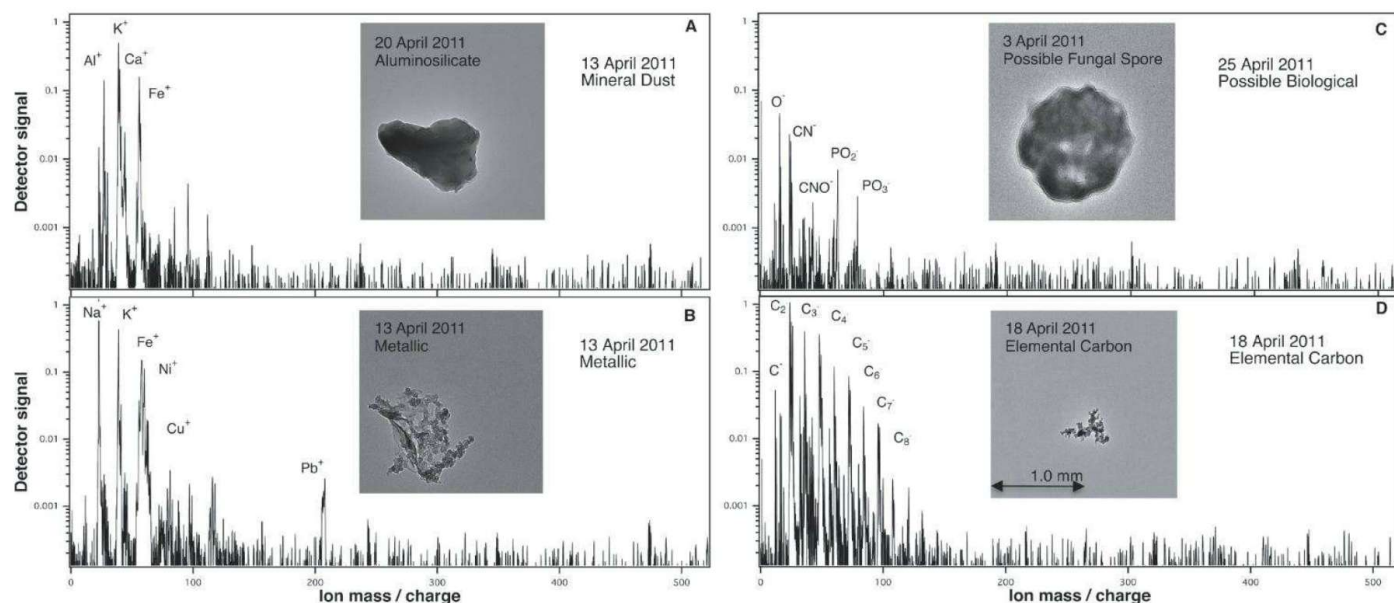


Fig. 2. Single-particle MS and EM (inset) images of IR particles, analyzed during the MACPEX aircraft campaign, corresponding to the categories presented in Fig. 1. (A) MS: the most common residual mineral dust with minimal surface coating; EM: an aluminosilicate particle, also without apparent surface coating. **(B)** MS: metallic particle with sodium, potassium, nickel, copper, iron, and lead; EM: metallic particle with tin, carbon, and silicon. **(C)** MS: one of six particles of possible biological origin

suggested by a composition including carbon, nitrogen, oxygen, and phosphorous (12); EM: the only particle of possible biological origin found on the electron microscope grids suggested by a composition including carbon, hydrogen, oxygen, and phosphorous. **(D)** MS: an EC particle; EM: one of two EC particles, identified by both composition and fractal spherule aggregate structure. Note that the spectra and images do not correspond to the same particles.

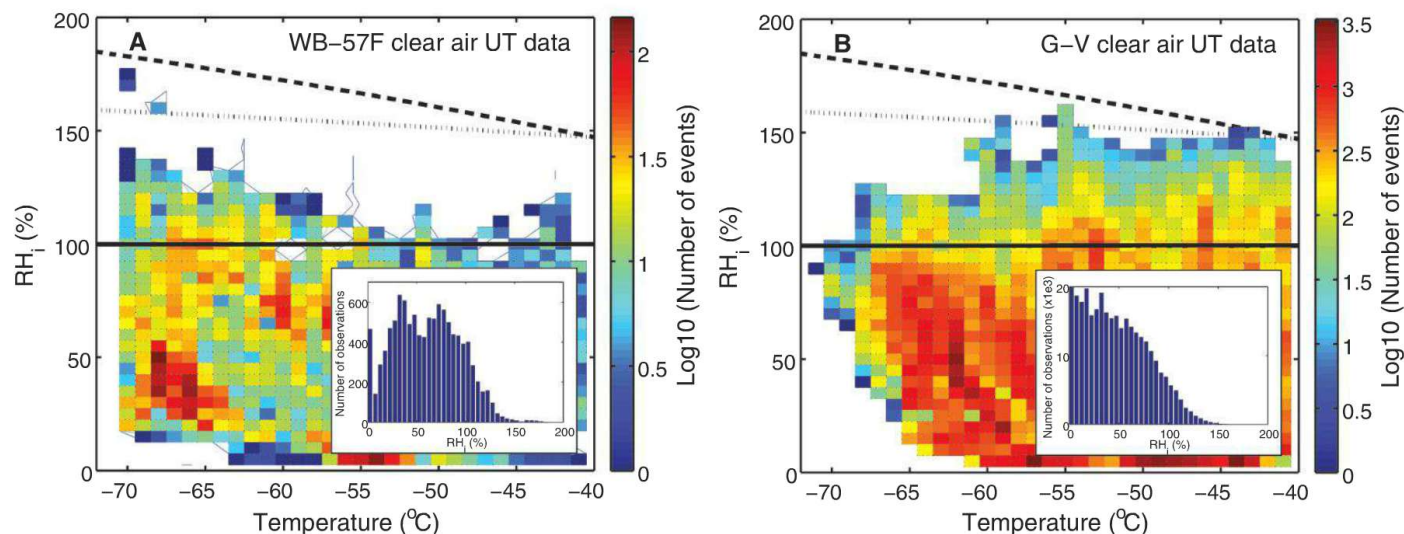


Fig. 3. Distribution of upper tropospheric, clear-air relative humidity with respect to ice (RH_i) as a function of temperature. Color-coding corresponds to the number of events observed in each 5% RH_i and 1°C bin. The dashed and solid lines indicate ice and liquid water saturation, respectively. **(A)** Composite of data collected in conjunction with the IR data shown in Figs. 1 and 2. The inset depicts a histogram of these data for temperatures warmer than -70°C .

Greater than 99% of the RH_i data in this temperature range fall below the threshold for homogeneous nucleation, denoted by the dotted lines. **(B)** Composite of data collected over a larger geographical area onboard the NSF G-V aircraft. The RH_i data account for water vapor mixing ratios measured by the Harvard Water Vapor instrument (17) and the Vertical Cavity Surface Emitting Laser hygrometer (18). Inset to (B), same as in (A). UT, universal time.

threshold for homogeneous freezing, also suggesting that heterogeneous freezing is the dominant formation mechanism.

Because ice-crystal number densities ranging from 1s to 100s per liter are consistent with heterogeneous freezing, whereas larger abundances are indicative of homogeneous nucleation (19), these measurements offer a third constraint on ice-formation mechanisms. Recently realized artifacts associated with ice-crystal shattering and consequent overcounting of number density render many previous conclusions uncertain. Higher-confidence data from the most recent set of flights indicate that only a few percent of clouds have number densities greater than a few hundred per liter (19). The combination of in situ determination of IR composition, relative humidity measurements, and ice-crystal number densities from multiple field campaigns in different regions presents a compelling case to consider heterogeneous freezing the dominant mechanism of cirrus formation throughout the study regions. These data stand in contrast to recent model studies that suggest the opposite conclusion (7).

Mineral dust is ubiquitous in the atmosphere (20). In agreement with the data presented here, collections of snow and cloud samples have shown mineral dust to be the predominant atmospheric IN (4, 8, 14, 21, 22). Ground-based and laboratory studies that nucleate ice under controlled conditions also show mineral dust to be effective as IN at $<-10^{\circ}\text{C}$ and RH_i from 110 to 140% (3). Laboratory studies show that sulfate and organic surface coatings “deactivate” mineral dust particles, rendering them less effective as IN (23), consistent with our results. Analysis of mineral dust particles collected in the free troposphere shows that uncoated particles are present thousands of kilometers from their source (24). Our data show that the uncoated subset of mineral dust—likely composed of particles that have not undergone substantial aging or a cloud-processing event—is the most important. The climatological importance of mineral dust is consistently demonstrated by laboratory studies that characterize it as an effective ice nucleus, field studies that observe

its presence in the free troposphere, and IR analyses that show it dominates within ice crystals. In regions where the concentration of mineral dust and metallic aerosol is low, such as the high latitudes of the Southern Hemisphere, homogeneous freezing or ice nucleation by glassy particles and anhydrous salts (10, 11) may be of greater importance than observed in these studies.

The lack of elemental carbon (EC) and biological particles is notable. Neither was abundant in the near-cloud particles or the ice phase, representing $<1\%$ in all cases. The in situ results are supported by IR collection for offline electron microscopy (EM) compositional analysis (12, 21, 22) during the most recent set of flights. We observed only a single possible biological particle and two EC IR particles during these flights, representing $<<1\%$.

With few exceptions, laboratory studies of EC have shown these particles to be inefficient IN, with nucleation only at temperatures and supersaturations close to homogeneous freezing (9, 25). We conclude that effective ice-nucleating EC particles are of low abundance in the cirrus regime. However, this finding may not be valid in contrails, which were not studied. Our 2002 and 2011 measurements are noteworthy: wildfires injected EC- and biomass-burning particles into the upper tropospheric study region, yet these particles were not abundant as IRs. Thus, we suggest models ignore EC as an ice-forming particle type for cirrus clouds.

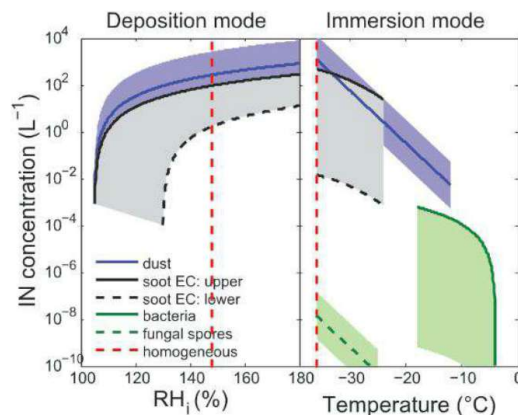
The case of biological particles is more complex than those of mineral dust and EC. Laboratory studies have shown that a few types of bacteria and fungal spores act as effective IN (9, 26). Field results, which include collections of ice-phase precipitation and residues from one orographic cloud, sometimes show the presence of biological material (27). Particle-phase scavenging is a greater concern in precipitation studies than in those of cirrus clouds, but nonetheless, these results suggest that a subset of biological particles are effective IN. The upper tropospheric data set reported here does not support an abundance of biological material as IRs, widespread

internal mixing with mineral dust, or the simple identification suggested in other studies (12, 27). This data set suggests that most biological particles generated at Earth’s surface are removed via dry or wet deposition due to their large size and water- or ice-nucleating potential before they are transported to cirrus altitude. We recommend that models ignore biological material as a cirrus-forming particle type. The discrepancy between this and lower altitude studies suggests a fundamental difference between measurements made within or coupled to the boundary layer and those made in the mid- to upper troposphere. Furthermore, the lower-altitude IR data were obtained in a cloud with ice crystals considerably larger than those we have been able to sample without impactation artifacts (12), although even in that study, mineral dust residuals dominated over biological particles (27).

Metallic particles represent an IR particle type that has not been extensively studied in the laboratory. Our data include a diversity of species such as Pb, Zn, Sn, Cu, Ag, Mo, and other heavy metals that have low abundance in mineral dust (Fig. 2). These metals were present in elemental, oxide, and sulfate forms. During the most recent set of flights, metallic particles represented 9 to 26% of the IRs. A few previous field studies have noted metal-rich “industrial” IN (4, 8, 28, 29). Lead, present in $\sim 5\%$ of ambient particles (30), has been shown to be a particularly efficient ice nucleus in the field and the laboratory (21, 29). Laboratory studies and improved emission inventories coupled to cloud-formation models are needed to elucidate the global effect of anthropogenic particles on cirrus clouds.

Both the number and activity of heterogeneous IN control the formation of cirrus clouds. A global simulation of upper tropospheric aerosol-particle concentrations combined with laboratory measurements of IN were used to predict cirrus formation. Independent of the IR composition measurements, this analysis also asserts the dominance of mineral dust among cirrus IN (9, 12, 31, 32) (Fig. 4). In both depositional- and immersion-freezing modes, mineral dust contributes the largest number of free tropospheric IN. Laboratory studies of EC suggest a wide range of IN efficiencies. In only the extreme case where all EC is assumed to have the highest efficiency does this species rival mineral dust. Biological particles are shown to be extremely rare in the upper troposphere, rendering them unable to compete with mineral dust in the cirrus temperature range ($<-30^{\circ}\text{C}$). Although many air parcels originating from mineral-dust emission areas undergo cloud processing before reaching cirrus altitudes (20), resulting in depletion and/or coating of these particles, no comprehensive global data measurements of the aerosol coating state exist and, thus, are not included in this model. The scarcity of laboratory experiments on biomass burning and metallic particles render us unable to provide separate estimates for these species.

Fig. 4. Estimates of upper tropospheric IN concentration versus RH_i and temperature from global simulations of aerosol surface area concentration and ice nucleation active site (INAS) densities derived from various laboratory experiments (12). Estimates of upper tropospheric IN concentrations and annual average aerosol particle concentration from two versions of the CAM-Oslo model (31, 32) were used. These estimates are derived independently from the in situ data. Deposition (between -35° and -57°C) and immersion nucleation modes are considered separately. The onset of homogeneous freezing is indicated by the red dashed lines for an approximate freezing rate coefficient of $5 \times 10^{14} \text{ m}^{-3} \text{ s}^{-1}$. Shaded areas represent the range of measured INAS densities.



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Supplementary Materials

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Materials and Methods

Figs. S1 to S4

Tables S1 to S4

References (33–71)

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Epistasis Among Adaptive Mutations in Deer Mouse Hemoglobin

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Epistatic interactions between mutant sites in the same protein can exert a strong influence on pathways of molecular evolution. We performed protein engineering experiments that revealed pervasive epistasis among segregating amino acid variants that contribute to adaptive functional variation in deer mouse hemoglobin (Hb). Amino acid mutations increased or decreased Hb-O₂ affinity depending on the allelic state of other sites. Structural analysis revealed that epistasis for Hb-O₂ affinity and allosteric regulatory control is attributable to indirect interactions between structurally remote sites. The prevalence of sign epistasis for fitness-related biochemical phenotypes has important implications for the evolutionary dynamics of protein polymorphism in natural populations.

Nonadditive interactions between mutations (epistasis) can exert a strong influence on the rate and direction of evolutionary change (1, 2). Insights into mechanisms of epistasis between beneficial mutations can reveal the causes of constraints on adaptive protein evolution (3–10). Mechanisms of epistasis are often best revealed through detailed examinations of interactions between amino acid mutations in the same protein that contribute to variation in a measurable biochemical phenotype

(7, 9–15). Such studies are especially relevant to our understanding of evolutionary process when genetically based changes in the measured phenotype contribute to variation in fitness under natural conditions.

We investigated the nature of epistatic interactions between adaptive mutations in the hemoglobin (Hb) of deer mice (*Peromyscus maniculatus*). Deer mice that are native to high altitude have evolved an elevated Hb-O₂ affinity relative to lowland conspecifics (16–18), and this modification of protein function contributes to an adaptive enhancement of whole-animal physiological performance under hypoxia (19, 20). Comparisons between highland deer mice from the Rocky Mountains and lowland deer mice

from the Great Plains revealed genetic differences in Hb-O₂ affinity that are attributable to the independent or joint effects of 12 amino acid polymorphisms: 8 mutations in the α -chain subunits of the $\alpha_2\beta_2$ Hb tetramer, and 4 mutations in the β -chain subunits. These 12 amino acid polymorphisms exhibit pronounced altitudinal shifts in allele frequency, and population genetic analyses of nucleotide variation in the α - and β -globin genes revealed evidence for divergent selection between deer mouse populations that are native to different elevations (18, 21–23).

Structural variation in deer mouse Hb has a modular organization that reflects the linkage arrangement of the 12 amino acid polymorphisms. Within the α -chain subunit, five amino acid replacements are located in exon 2 of the underlying gene, and the remaining three replacements are located in exon 3. Polymorphic sites within the same exon are in nearly complete linkage disequilibrium (LD) with one another, but intragenic recombination has produced a partial uncoupling between the two exons (21, 23). The two most common α -globin allele classes are distinguished from each other by eight amino acid replacements at sites 50, 57, 60, 64, 71, 113, 115, and 116 (fig. S1A). The four amino acid polymorphisms in the β -globin gene are also in nearly complete LD with one another (18, 22). The two most common β -globin allele classes are distinguished from each other by four amino acid replacements at sites 62, 72, 128, and 135 (fig. S1B). Thus, in deer mouse populations, most of the naturally occurring variation in Hb

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structure is captured by combinatorial permutations of allelic variants at three loci: α -globin exon 2, α -globin exon 3, and β -globin.

We used site-directed mutagenesis to engineer all eight combinations of the α - and β -chain variants in recombinant Hb (rHb), and we measured O₂-binding properties of the purified proteins (24). In addition to the chimeric multipoint mutants, we also engineered 10 additional single- and double-mutant rHbs to measure the functional effects of specific point mutations individually and in pairwise combination. We synthesized rHbs representing the two most common variants from high- and low-altitude populations, designated “HH-H” and “LL-L,” respectively (the first two letters denote the separate α -chain subdomains encoded by exons 2 and 3, and the

third letter denotes the β -chain subunit). To test for epistasis, we also synthesized rHbs representing the remaining six combinations of H- and L-type alleles at each of the three loci (Fig. 1A). To examine variation in the allosteric regulation of Hb-O₂ affinity, we measured O₂-binding properties of each rHb mutant in the presence and absence of the two principal allosteric effectors present in mammalian red blood cells: Cl[−] ions and 2,3-diphosphoglycerate (DPG). These effectors reduce Hb-O₂ affinity by preferentially binding and stabilizing deoxyHb, thereby shifting the allosteric equilibrium in favor of the low-affinity T-state quaternary structure. By using standardized concentrations of Cl[−] and DPG in the physiological range, we ensured that in vitro measurements were relevant to in vivo conditions (24).

Our experiments revealed substantial variation in intrinsic Hb-O₂ affinity, as P_{50} values (the O₂ tension at 50% heme saturation) for stripped, cofactor-free rHbs ranged from 4.55 to 7.09 torr (Table 1). The high-altitude HH-H variant exhibited a 26% lower stripped P_{50} (i.e., higher intrinsic O₂ affinity) relative to the low-altitude LL-L variant (Table 1). Hb-O₂ affinity was reduced in the presence of Cl[−] ions (added as 0.1 M KCl), in the presence of DPG at a twofold molar excess over tetrameric Hb, and in the simultaneous presence of both effectors (Table 1). All rHbs exhibited cooperative O₂ binding, and Hill coefficients ranged from 1.36 to 2.28 in the presence of Cl[−] and DPG.

Contrary to the expectations of an additive null model, the phenotypic effects of allelic substitutions (L→H and H→L) at α -globin exon 2, α -globin exon 3, and β -globin were highly dependent on genetic background, as pairwise epistasis accounted for 40% of the variance in P_{50} values in the absence of allosteric effectors and 90% in the simultaneous presence of Cl[−] and DPG (Table 1). In the presence of both allosteric effectors, the HH α -globin allele conferred an increased affinity on the β L background and a decreased affinity on the β H background. Similarly, the H-type β -globin allele conferred an increased affinity on the α LL background and a decreased affinity on the α HH background. These are examples of sign epistasis (2), where the sign of the phenotypic effect of an allele is conditional on genetic background.

Because mammalian Hb is a heterotetramer ($\alpha_2\beta_2$), epistatic interactions could involve closely linked sites in the same gene or sites in unlinked genes that encode different subunits of the protein. Intragenic (within-subunit) epistasis could stem from localized modifications of secondary or tertiary structure, whereas intergenic (between-subunit) epistasis could stem from allosteric transitions in quaternary structure between different oxygenation states of the Hb tetramer. Epistasis for Hb-O₂ affinity is mainly attributable to the suppressed DPG sensitivity of chimeric rHb variants that incorporate the products of α - and β -globin alleles of unlike type (α HH combined with β L, and vice versa; Table 1 and Fig. 1B). Although DPG sensitivity was suppressed in

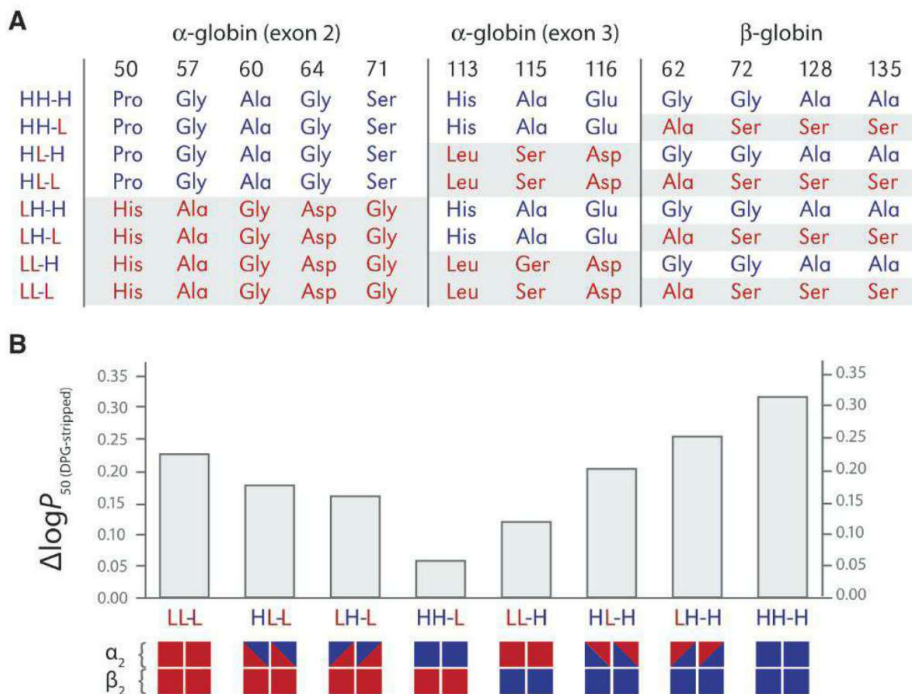


Fig. 1. Structural and functional variation among recombinant deer mouse Hbs (rHbs). (A) rHbs representing all combinatorial permutations of allelic variants at α -globin exon 2, α -globin exon 3, and β -globin. Shaded regions represent the products of low-altitude L-type alleles (red), and unshaded regions represent products of high-altitude H-type alleles (blue). (B) Variation in the allosteric regulation of Hb-O₂ affinity by DPG. Sensitivity to DPG is indexed by the difference in log-transformed P_{50} values between stripped Hb in the presence and absence of DPG.

Table 1. O₂ affinities (P_{50} , torr; mean $\pm$ SEM) and allosteric properties of purified rHbs. Sensitivities to allosteric effectors are measured as the difference in log-transformed P_{50} values in the presence and absence of each effector, individually and in combination. V_A and V_E are estimated components of additive and epistatic variance, respectively (24).

	LL-L	HL-L	LH-L	HH-L	LL-H	HL-H	LH-H	HH-H	V_A	V_E
P_{50} (torr)										
stripped	5.72 $\pm$ 0.23	6.37 $\pm$ 0.05	7.09 $\pm$ 0.55	6.59 $\pm$ 0.11	5.25 $\pm$ 0.09	5.49 $\pm$ 0.04	6.32 $\pm$ 0.15	4.55 $\pm$ 0.08	0.597	0.403
+KCl	10.20 $\pm$ 0.03	12.55 $\pm$ 0.41	10.83 $\pm$ 0.78	12.88 $\pm$ 0.27	12.15 $\pm$ 0.31	11.18 $\pm$ 0.30	12.01 $\pm$ 0.20	9.70 $\pm$ 0.47	0.051	0.949
+DPG	9.72 $\pm$ 0.17	9.64 $\pm$ 0.12	10.33 $\pm$ 0.20	7.54 $\pm$ 0.25	6.93 $\pm$ 0.19	8.84 $\pm$ 0.26	11.44 $\pm$ 0.30	9.53 $\pm$ 0.27	0.189	0.811
+KCl + DPG	10.46 $\pm$ 0.30	11.21 $\pm$ 0.33	12.51 $\pm$ 0.59	8.63 $\pm$ 0.21	7.65 $\pm$ 0.22	11.36 $\pm$ 0.34	12.83 $\pm$ 0.34	10.13 $\pm$ 0.35	0.096	0.904
$\Delta \log P_{50}$										
KCl – stripped	0.251	0.295	0.184	0.291	0.364	0.309	0.279	0.329	0.671	0.329
DPG – stripped	0.230	0.180	0.163	0.058	0.121	0.206	0.258	0.321	0.726	0.274
(KCl + DPG) – stripped	0.262	0.246	0.247	0.117	0.164	0.316	0.308	0.348	0.214	0.786

HH-L and LL-H, allosteric regulatory capacities of the chimeric rHbs were partially restored by reciprocally converting either of the two α -chain subdomains to the type that matched the associated β -chain subunit: DPG sensitivity of the chimeric LL-H was partially restored by L $\rightarrow$ H substitutions at α -globin exon 2 or exon 3, and reciprocally, DPG sensitivity of the chimeric HH-L was partially restored by H $\rightarrow$ L substitutions at these same loci (Fig. 1B). In principle, a suppressed DPG sensitivity (and hence, increased Hb-O₂ affinity) could be produced by charge-changing amino acid replacements that eliminate phosphate-binding sites in the β -chain subunits. Because the positively charged phosphate-binding sites are invariant in deer mouse β chains (17, 18), allelic variation in DPG sensitivity must stem from indirect, second-order perturbations.

Analysis of the crystal structure of deer mouse Hb at 1.8 Å resolution (24, 25) revealed that each of the eight rHb mutants is characterized by a unique constellation of hydrogen bonds within and between subunits (Table 2

and fig. S2). Additional hydrogen bonds between subunits of the same $\alpha\beta$ dimer are formed in the presence of β 128Ser (an L-type residue; fig. S2), which contributes to the observed epistasis between allelic α - and β -chain variants. Structural analysis also revealed that in Hbs with L-type α -globin, the imidazole ring of α 50His forms a hydrogen bond with α 30Glu in the same subunit. The replacement of α 50His with Pro (the H-type residue) eliminates this hydrogen bond and causes a subtle reorientation of the E helix and CD loop (Fig. 2), an effect that propagates to the α 1 β 2 intersubunit contact and shifts the allosteric equilibrium in favor of the high-affinity oxyHb (R-state) quaternary structure.

To test the effects of charge-changing α -chain mutations in the CD loop (α 50His/Pro) and the adjacent E helix (α 64Asp/Gly), we synthesized each of the alternative single and double mutants on both HH-H and LL-L backgrounds. The experiments revealed that, on the LL-L background, substitutions of H-type residues (α 50His $\rightarrow$ Pro and α 64Asp $\rightarrow$ Gly) did not produce a significant

increase in Hb-O₂ affinity individually or in combination; however, on the HH-H background, single-step reversions to L-type residues at both sites produced significant reductions in Hb-O₂ affinity in the presence of allosteric effectors (fig. S3). We also measured the individual effects of all four amino acid mutations in the β -chain subunit. On the LL-L background, the substitution β 128Ser $\rightarrow$ Ala, which removes an α 1 β 1 hydrogen bond (fig. S2), produced an increased anion sensitivity (and hence, a decreased Hb-O₂ affinity in the presence of Cl⁻ and DPG; fig. S4). However, on this same background, introducing all four H-type β -chain mutations in combination produced a highly significant increase in Hb-O₂ affinity in the presence of allosteric effectors (fig. S4).

In summary, results of our mutagenesis experiments revealed pervasive epistasis among segregating amino acid variants in deer mouse Hb (Table 1). The individual and joint effects of α - and β -chain point mutations contribute to the elevated Hb-O₂ affinity of highland deer mice, but the effects of these mutations are highly dependent on the allelic state of other residue positions.

Directed mutagenesis studies have unveiled “cryptic” epistasis between amino acid substitutions that distinguish deeply diverged orthologous proteins (7, 11, 13, 14). Similarly, experimental studies of microbial systems have revealed intragenic epistasis between sites that underwent successive allelic substitutions but that were never simultaneously polymorphic (6, 10). By contrast, the interacting mutations in deer mouse Hb are segregating in natural populations and, given the extensive intragenic and intergenic LD, the epistasis contributes to additive genetic variance in Hb function, providing an explanation for the previously documented variation in anion sensitivity of deer mouse Hbs (17, 18). Given the evidence for spatially varying selection on Hb polymorphism in relation to altitude, the pervasiveness of sign epistasis for Hb-O₂ affinity suggests that the selection coefficient for a given allele will often be highly dependent on the allelic composition of the local population. Thus, sign epistasis among segregating amino acid variants may exert a strong influence on allele frequency dynamics and mutational pathways of protein evolution.

Table 2. Allelic variation in the network of atomic contacts within and between subunits of deer mouse Hb. Plus signs denote the presence of hydrogen bonds within subunits (α 50His- α 30Glu and α 113His- α 24Tyr) or between subunits of unlike type (α 34Cys- β 128Ser). Polymorphic sites are shown in bold.

	LL-L	HL-L	LH-L	HH-L	LL-H	HL-H	LH-H	HH-H
H-bonds								
α 50His- α 30Glu	+		+		+		+	
α 113His- α 24Tyr			+	+			+	+
α 34Cys- β128Ser	+	+	+	+				

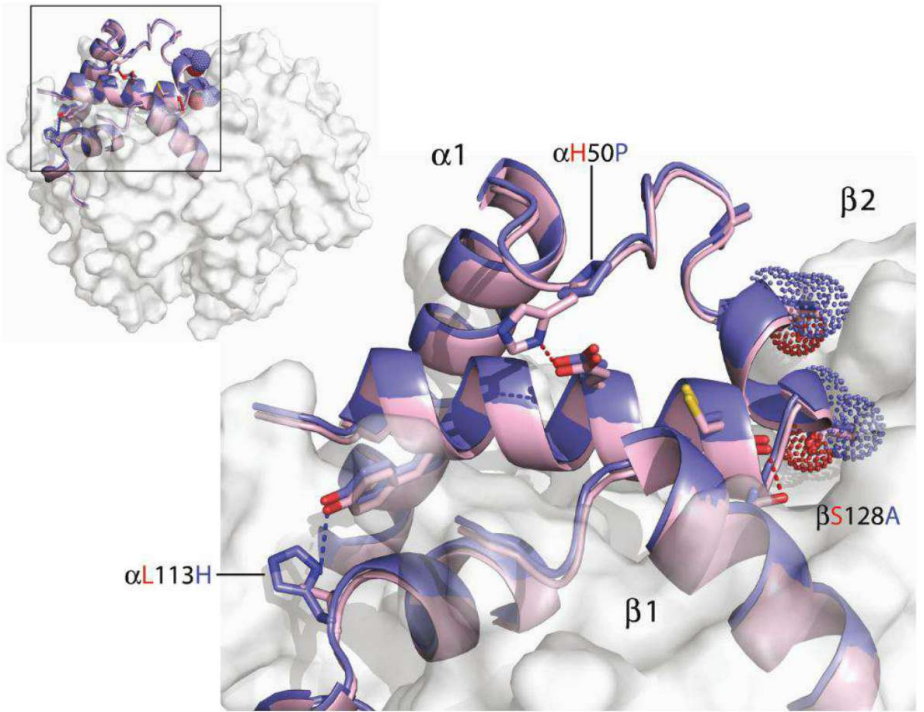


Fig. 2. Difference in the network of hydrogen bonds between high- and low-altitude Hb variants, HH-H and LL-L, respectively. The α 1 and β 1 subunits of HH-H (light blue) and LL-L (light red) are superimposed, and van der Waals radii are shown for α -chain residues that are in atomic contact with β -chain residues of the opposing α 2 β 2 dimer.

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Supplementary Materials

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Figs. S1 to S5

Table S1

References (26–41)

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Root Effect Hemoglobin May Have Evolved to Enhance General Tissue Oxygen Delivery

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The Root effect is a pH-dependent reduction in hemoglobin-O₂ carrying capacity. Specific to ray-finned fishes, the Root effect has been ascribed specialized roles in retinal oxygenation and swimbladder inflation. We report that when rainbow trout are exposed to elevated water carbon dioxide (CO₂), red muscle partial pressure of oxygen (PO₂) increases by 65%—evidence that Root hemoglobins enhance general tissue O₂ delivery during acidotic stress. Inhibiting carbonic anhydrase (CA) in the plasma abolished this effect. We argue that CA activity in muscle capillaries short-circuits red blood cell (RBC) pH regulation. This acidifies RBCs, unloads O₂ from hemoglobin, and elevates tissue PO₂, which could double O₂ delivery with no change in perfusion. This previously undescribed mechanism to enhance O₂ delivery during stress may represent the incipient function of Root hemoglobins in fishes.

In vertebrates, hemoglobin (Hb) plays a crucial role in optimizing tissue oxygen (O₂) delivery by increasing blood O₂-carrying capacity and regulating the partial pressure (PO₂) at which O₂ is delivered. Within tissues (such as muscle), metabolically produced carbon dioxide (CO₂) reduces blood pH and thus Hb-O₂ affini-

ty, elevating blood PO₂ and enhancing O₂ delivery, which is collectively termed the Bohr effect (1). In mammals, this may elicit an increase in blood PO₂ of up to 2 mmHg (2, 3) in vivo, providing some 5% increase in O₂ delivery. Teleost fishes often have much more pH-sensitive Hbs, and a recent in vitro study indicates that this ef-

fect may be an order of magnitude greater (4). The current study confirms this in vivo. Enhanced O₂ delivery may represent an important step in the extraordinary adaptive radiation of the teleost fishes (5), which make up almost half of all vertebrate species.

In teleosts, a reduction in blood pH reduces both Hb-O₂ affinity and O₂ carrying capacity, which is known as the Root effect (6) and has a well-studied role in securing O₂ delivery to the retina and swimbladder (7, 8). These tissues possess specialized acid-producing cells in conjunction with a dense counter-current capillary network (*rete*) that localizes and magnifies a large acidosis, thus promoting O₂-offloading via the Root effect (7, 8). This system is effective enough to generate O₂ tensions exceeding 50 atm (~38,000 mmHg) within the gas-filled swimbladder (7).

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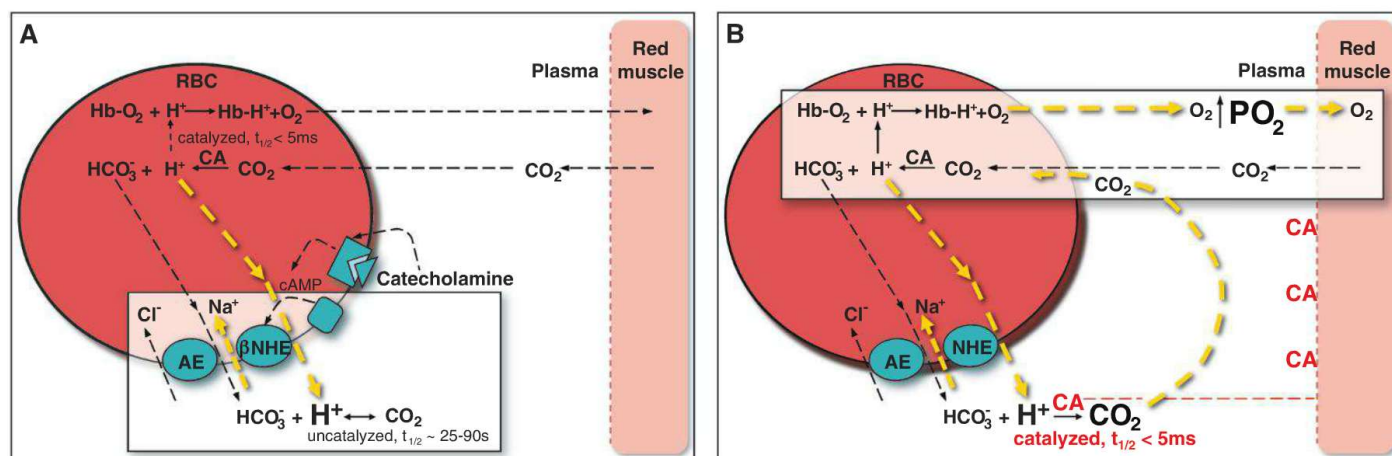


Fig. 1. (A and B) Schematic representation of a catecholamine-activated RBC pH disequilibrium (A) short-circuited by plasma-accessible CA to elevate tissue PO₂ (B). AE, anion exchange; cAMP, adenylyl cyclase and 3',5'-cyclic monophosphate.

The Root effect could, however, be a liability because it could severely limit O_2 uptake at the gills if systemic blood becomes too acidic. Many teleosts release stress hormones (noradrenaline and adrenaline) that stimulate RBC Na^+/H^+ exchange (β NHE), which removes H^+ in exchange for Na^+ , creating a pH disequilibrium across the RBC membrane (Fig. 1A) (9). This increases RBC pH and ensures O_2 loading at the gill during acidotic stress associated with exhaustive exercise, hypoxia, or hypercarbia (Fig. 1A) (10, 11). Carbonic anhydrase (CA), which catalyzes the reversible conversion of HCO_3^- and H^+ to CO_2 for diffusive excretion, would rapidly short-circuit this process (Fig. 1B) (4, 12). Consequently, there is no plasma-accessible CA in the gills of teleosts (13), and this is understood to ensure O_2 -loading during stressful conditions. However, there is evidence for plasma-accessible CA in muscle capillaries (14, 15). This would short-circuit β NHE, reduce RBC pH and Hb- O_2 affinity, and enhance O_2 -unloading and tissue delivery (Fig. 1B). A recent in vitro study provided proof of principle that this can occur; exposure of acidified and adrenergically stimulated rainbow trout blood to CA elevated PO_2 by over 30 mmHg (4, 12). Thus, plasma-accessible CA may allow Root effect Hbs to enhance O_2 delivery to aerobic tissues during acidotic stress (4, 12). The present study was designed specifically to determine whether plasma-accessible CA and the associated elimination of RBC pH disequilibrium states in capillary beds could markedly increase tissue PO_2 and the driving force for O_2 delivery in vivo.

We implanted fiber optic O_2 sensors into rainbow trout red muscle (RM) (fig. S1) to monitor RM PO_2 in real-time. We then monitored changes in RM PO_2 during a mild acidosis elicited by hypercarbia (elevated water CO_2), which should enhance the RBC pH disequilibrium at the gills. We hypothesized that if present in the RM capillary endothelium, plasma-accessible CA would abolish the pH disequilibrium and increase RM PO_2 . To provide evidence for a role of plasma-accessible CA in this phenomenon, a new membrane-impermeant CA inhibitor, C18 [compound 18, laboratory-synthesized according to (16)] (fig. S2), was injected into the bloodstream so as to abolish the hypercarbic increase in RM PO_2 .

After anesthesia, dorsal aorta (DA) cannulation, and RM O_2 sensor implantation (fig. S1) (17), rainbow trout recovered overnight in a black Perspex chamber while RM PO_2 was continuously monitored. In the morning, blood was sampled, and fish were lightly anaesthetized and treated with a skeletal muscle relaxant (tubocurarine) so as to prevent movement of the fish and damage to the O_2 sensor during hypercarbia. All blood variables recovered by 90 ± 21 min after tubocurarine, which represented “baseline” conditions against which subsequent responses were compared (Fig. 2 and fig. S3).

Fish were force-ventilated to ensure that arterial PO_2 (P_{aO_2} ; 111.1 ± 14.9 mmHg), Hb- O_2

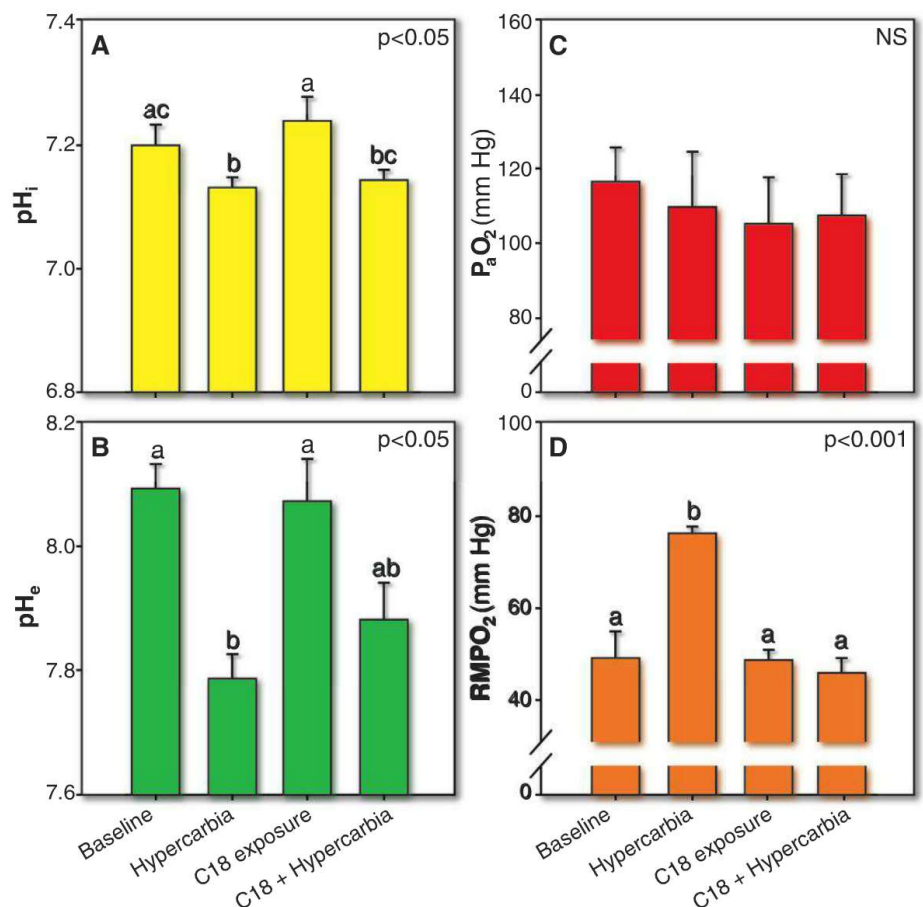


Fig. 2. The effect of hypercarbia, membrane-impermeant carbonic anhydrase inhibitor (C18) and combined hypercarbia and C18 on (A) pH_i , (B) pH_e , (C) P_{aO_2} , and (D) RM PO_2 . Different lowercase letters demarcate significant differences between treatment groups within a panel. All data are presented as means $\pm$ SEM, $n = 7$ to 11 fish per treatment per treatment. Statistical significance (repeated measures analysis of variance) is indicated with P values (nonsignificance is indicated “NS”) and assessed using $\alpha < 0.05$.

saturation (86.8 to 99.0%), and O_2 content (7.1 to 7.7 ml O_2 100 ml blood $^{-1}$) remained constant (Fig. 2 and fig. S3). Plasma catecholamines (noradrenaline, NA; adrenaline, AD) were not significantly elevated over published resting values (≤ 12 and ≤ 9 nM, respectively) (fig. S4) (18).

Hypercarbia caused a significant increase in blood P_{CO_2} from 1.7 ± 0.1 to 4.0 ± 0.3 mmHg [$F(5, 10) = 8.20$, $P < 0.001$] but no significant change in RBC or plasma total CO_2 (T_{CO_2}) (table S1). The elevated P_{CO_2} elicited a significant decrease in intracellular RBC pH (pH_i) (7.23 ± 0.01 to 7.13 ± 0.02) and extracellular (plasma) pH (pH_e) (8.09 ± 0.04 to 7.79 ± 0.04) (Fig. 2 and fig. S3). There was a 65% increase in RM PO_2 from 47.1 ± 6.4 to 75.2 ± 2.2 mmHg, a ΔPO_2 of 30.9 ± 7.6 mmHg (Figs. 2 and 3 and figs. S3 and S4). Upon return to normocarbica, all physiological variables returned to baseline (Fig. 2, figs. S3 and S4, and table S1). Intra-arterial C18 injection during normocarbica decreased [Hb] and Hct with no significant effects on blood, plasma, or RM variables (Fig. 2, figs. S3 and S5, and table S1). Upon reexposure to hypercarbia (C18+hypercarbia), blood P_{CO_2}

[$F(5, 10) = 8.20$, $P < 0.001$] and RBC T_{CO_2} [$F(5, 10) = 3.88$, $P < 0.001$] both increased, with plasma T_{CO_2} unaffected (table S1). pH_i decreased to 7.14 ± 0.02 [$F(5, 8) = 4.62$, $P < 0.01$], but no changes in pH_e were observed (Fig. 2 and fig. S3). After C18 treatment, however, hypercarbia did not elicit the significant change in RM PO_2 that had previously been observed [$\Delta PO_2 = 0.01 \pm 2.96$ mmHg; $t(15) = 3.96$, $P = 0.001$] (Fig. 2 and figs. S3 and S5).

Our findings support the hypothesis that in a teleost fish possessing a Root effect Hb and plasma-accessible CA, O_2 delivery to tissues other than the retina and swimbladder—specifically, RM—can be greatly enhanced during acidotic stress. In RM, the proposed mechanism is a cascade by which plasma-accessible CA at the tissue eliminates an arterial RBC pH disequilibrium state to acidify RBCs. This causes Hb- O_2 unloading and elevates RM PO_2 (Fig. 1B). The level of hypercarbia we used caused only a moderate acidosis but was associated with a profound increase in RM PO_2 over baseline. This effect on RM PO_2 was abolished by the membrane-impermeant CA inhibitor (C18), highlighting the

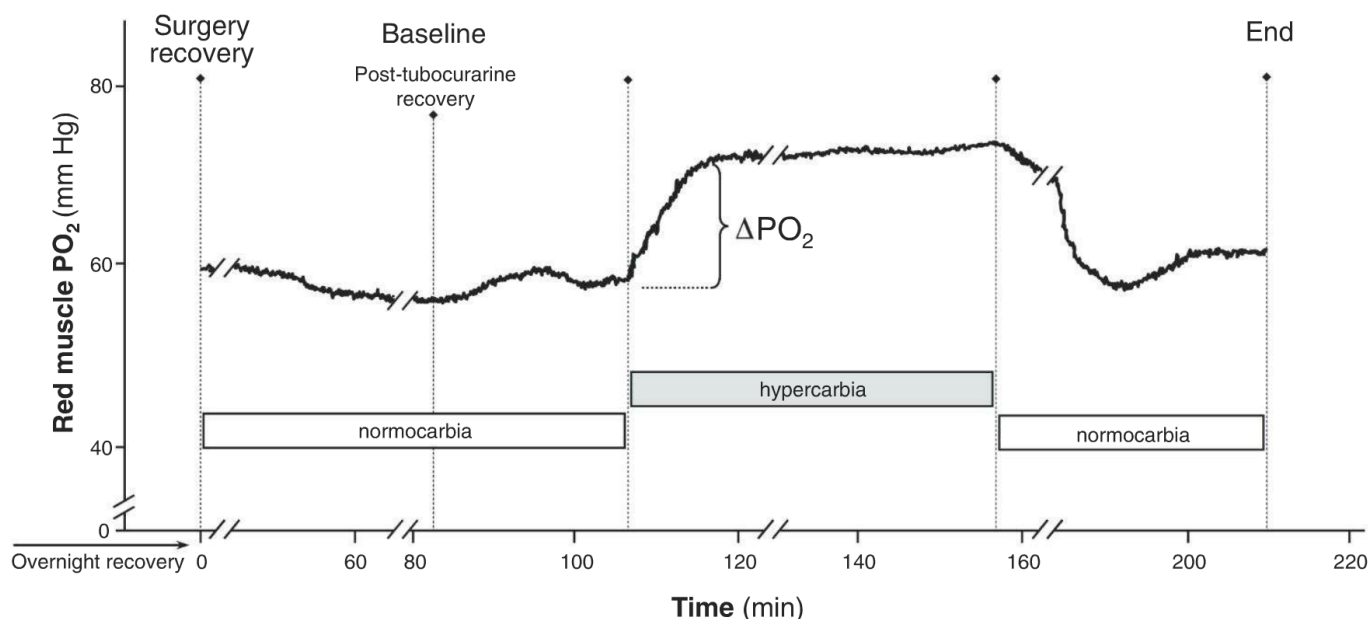


Fig. 3. Representative trace illustrating on-line changes in rainbow trout RM PO_2 (ΔPO_2) upon exposure to hypercarbia. The y axis represents RM PO_2 (mmHg), and the x axis indicates elapsed time (min). Vertical lines indicate experimental treatments.

importance of plasma-accessible CA in the response. We estimate that a ΔPO_2 of this magnitude could almost double tissue O_2 delivery with no change in perfusion (supplementary text and fig. S8). Exposure to this level of hypercarbia did not significantly elevate catecholamine levels, indicating that the treatment was not overly stressful, and activation of RBC β NHE may not have been the prime source of the pH disequilibrium (4); other stimuli may activate the β NHE (19, 20), or additional RBC NHE isoforms may exist. Activation of the RBC β NHE under more stressful conditions, such as exercise or hypoxaemia, may have an even greater influence on tissue oxygenation. This may be particularly important to increase aerobic metabolism during environmental or exercise stress or to speed up recovery after an intense bout of exercise—for example, after a predator-prey encounter or during long, upstream migrations, as exhibited in Pacific salmon.

It has been suggested that the Bohr effect evolved independently three times in vertebrates, but only once was this associated with the Root effect (21, 22). According to the mechanism we propose [both here and in vitro (4)], the larger Bohr associated with the Root effect could actually promote O_2 delivery in teleosts. Thus, in response to previous arguments (23), a large Bohr coefficient could have been selected for without compromising general O_2 delivery. For this to be the case, however, three components are required: (i) pH-sensitive Hbs, such as Root effect Hbs; (ii) plasma-accessible CA in the respective capillary beds but not at the gill (4); and (iii) RBC β NHE or NHE activity to generate a pH disequilibrium in the absence of CA. All of these traits may depend on phylogeny, life history, and/or lifestyle of the species. With these components in place, however, a Root

effect Hb can greatly enhance tissue O_2 delivery during a relatively mild acidosis. Further studies are required to investigate the degree to which this occurs in other metabolically active tissues, other species, and ultimately, the role that this mechanism of enhanced oxygen delivery may have played in the extraordinary success of teleosts.

Root effect Hbs evolved in ray-finned fishes ~400 million years ago, at least 150 million years before the appearance of the choroid rete at the eye or *rete mirabile* at the swimbladder, the structures that are generally associated with this exceptional O_2 delivery system (21, 22). Here, we provide in vivo evidence supporting the hypothesis that Root effect Hbs, in the presence of plasma-accessible CA, can greatly enhance systemic tissue O_2 delivery during periods of acidotic stress. We estimate that the 65% elevation in RM PO_2 would almost double tissue O_2 delivery with no change in perfusion. The role of Root effect Hbs in the eye and swimbladder may, therefore, be an exaptation, as proposed by Berenbrink *et al.* (22). That is, an incipient function of Root Hbs—general O_2 delivery—may have been co-opted to give rise to the complex physiological system at the eye and swimbladder 150 million to 270 million years later.

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Supplementary Materials

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Targeting Isoprenylcysteine Methylation Ameliorates Disease in a Mouse Model of Progeria

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Several progeroid disorders, including Hutchinson-Gilford progeria syndrome (HGPS) and restrictive dermopathy (*ZMPSTE24* deficiency), arise when a farnesylated and methylated form of prelamin A accumulates at the nuclear envelope. Here, we found that a hypomorphic allele of isoprenylcysteine carboxyl methyltransferase (ICMT) increased body weight, normalized grip strength, and prevented bone fractures and death in *Zmpste24*-deficient mice. The reduced ICMT activity caused prelamin A mislocalization within the nucleus and triggered prelamin A-dependent activation of AKT-mammalian target of rapamycin (mTOR) signaling, which abolished the premature senescence of *Zmpste24*-deficient fibroblasts. ICMT inhibition increased AKT-mTOR signaling and proliferation and delayed senescence in human HGPS fibroblasts but did not reduce the levels of misshapen nuclei in mouse and human cells. Thus, targeting ICMT might be useful for treating prelamin A-associated progeroid disorders.

Children with Hutchinson-Gilford progeria syndrome (HGPS) exhibit premature aging phenotypes and often die during their teenage years. HGPS is caused by mutations in the gene encoding prelamin A and lamin C (*LMNA*) that result in an internally truncated form of prelamin A (called progerin) that accumulates at the nu-

clear rim and induces nuclear shape abnormalities (1–4). Progerin retains its carboxyl-terminal *CAAX* motif, which triggers farnesylation of the cysteine (i.e., the “C” in the *CAAX* motif) by protein farnesyltransferase (FTase) (fig. S1). Interfering with farnesylation with an FTase inhibitor (FTI) reduces the frequency of misshapen

nuclei in *Zmpste24*-deficient fibroblasts and HGPS fibroblasts and ameliorates disease phenotypes in *Zmpste24*-deficient mice and other models of HGPS, although the benefits are modest (2, 5–10). A recent clinical trial of an FTI in children with HGPS showed a modest beneficial effect on disease phenotypes (11, 12).

After farnesylation, the last three amino acids (the “AAX” of the *CAAX* motif) of prelamin A and progerin are clipped off, and the farnesylcysteine is methylated by isoprenylcysteine carboxyl methyltransferase (ICMT) (fig. S1). Methylation of some *CAAX* proteins, including the RAS oncoprotein, is important for proper targeting to membranes within cells (13, 14), but the relevance of methylation to prelamin A-associated progeroid disorders is unknown. To address this issue, we took advantage of a hypomorphic *lcm*t allele (*lcm*t^{hm}), fortuitously created by the insertion of *loxP* sites flanking exon 1 of *lcm*t (14, 15). *lcm*t^{hm/hm} mice were leaner than *lcm*t^{hm/+} littermates (fig. S2,

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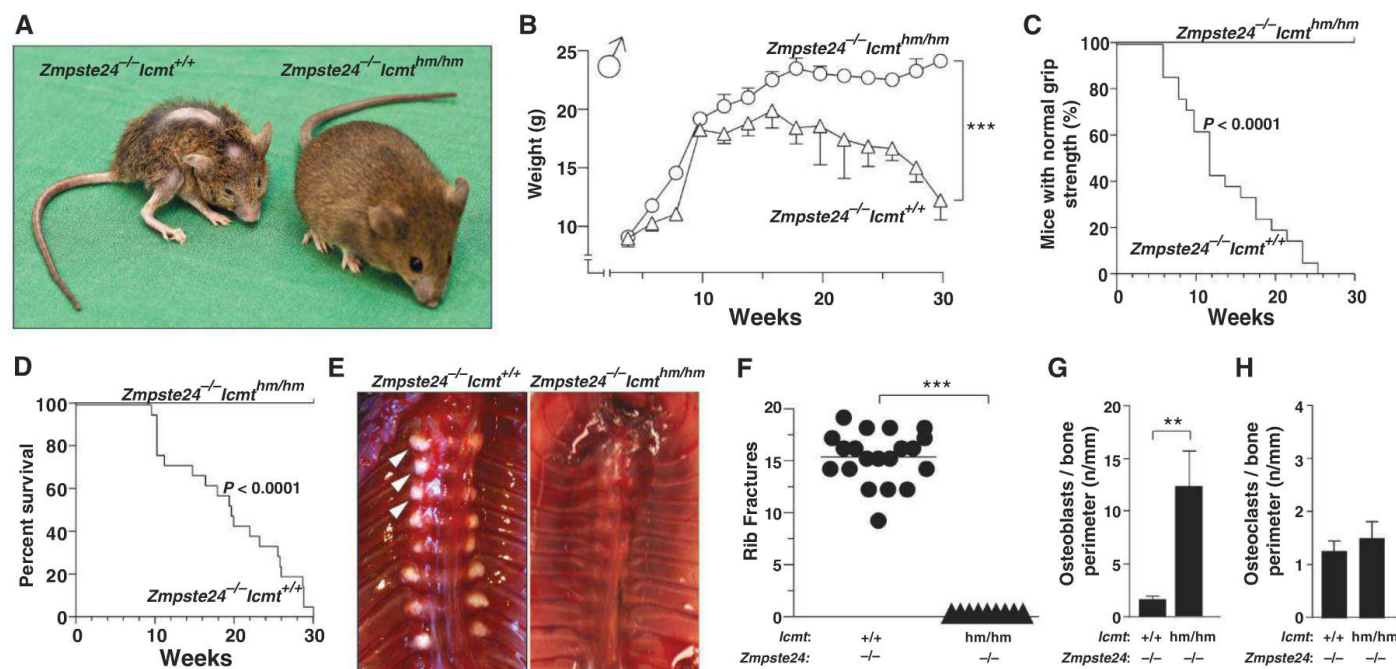


Fig. 1. Targeting *lcm*t ameliorates disease phenotypes and prevents death in 30-week-old *Zmpste24*^{-/-} mice. (A) Photograph of 24-week-old littermate mice. (B) Body-weight curves of male *Zmpste24*^{-/-}*lcm*t^{+/+} (*n* = 11) and *Zmpste24*^{-/-}*lcm*t^{hm/hm} (*n* = 5) mice. (C) Kaplan-Meier plot showing the percentage of *Zmpste24*^{-/-}*lcm*t^{+/+} (*n* = 21) and *Zmpste24*^{-/-}*lcm*t^{hm/hm} (*n* = 9) mice with normal grip strength. (D) Kaplan-Meier plot showing survival of *Zmpste24*^{-/-}*lcm*t^{+/+}

(*n* = 21) and *Zmpste24*^{-/-}*lcm*t^{hm/hm} (*n* = 9) mice. (E) Ventral view of spinal columns from an 18-week-old *Zmpste24*^{-/-}*lcm*t^{+/+} mouse and a 30-week-old *Zmpste24*^{-/-}*lcm*t^{hm/hm} mouse. Arrowheads indicate rib fractures at costovertebral joints. (F) Number of rib fractures in *Zmpste24*^{-/-}*lcm*t^{+/+} (*n* = 21) and *Zmpste24*^{-/-}*lcm*t^{hm/hm} (*n* = 9) mice. (G and H) Cellular parameters of L2 vertebrae (*n* = 6 per genotype). ***P* < 0.01; ****P* < 0.001. Data are presented as mean ± SEM.

A to D) but were healthy and lived for >2 years. The reduced ICMT expression in *Icmt*^{hm/hm} cells inhibited prelamin A processing to lamin A (fig. S2E).

We bred *Zmpste24*^{-/-} mice harboring the *Icmt*^{hm} allele. ICMT expression and activity levels were 70 to 90% lower in *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice than in *Zmpste24*^{-/-}*Icmt*^{+/+} littermates (fig. S3, A to D). Incubating *Zmpste24*^{-/-}*Icmt*^{hm/hm} fibroblasts with a Cre-adenovirus (*adCre*) eliminated ICMT expression (fig. S3, B and D). The reduced ICMT activity in *Zmpste24*^{-/-}*Icmt*^{hm/hm} livers resulted in a moderate accumulation of ICMT substrates (fig. S3E). To determine whether prelamin A existed in unmodified form in *Zmpste24*^{-/-}*Icmt*^{hm/hm} cells, we performed mass spectrometry of prelamin A in isolated fibroblast nuclei (fig. S3F). The relative levels of unmodified prelamin A were higher in nuclei of *Zmpste24*^{-/-}*Icmt*^{hm/hm} and *Zmpste24*^{-/-}*Icmt*^{Δ/Δ} cells than in *Zmpste24*^{-/-}*Icmt*^{+/+} cells (fig. S3G).

As expected, *Zmpste24*^{-/-}*Icmt*^{+/+} mice developed alopecia, a hobbling gait, growth retardation, and reduced grip strength; they began to die at ~10 weeks of age (16–18) (Fig. 1, A to D, and fig. S4). By 30 weeks, all had died or been euthanized (Fig. 1D). At that time, *Zmpste24*^{-/-}*Icmt*^{+/+} mice had multiple rib fractures adjacent to the costovertebral joints (Fig. 1, E and F). L2 ver-

tebrae from *Zmpste24*^{-/-}*Icmt*^{+/+} mice exhibited osteopenia and reduced osteoid, suggesting little or no ongoing bone formation (fig. S5A). In contrast, all *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice were alive at 30 weeks, and their gait, grip strength, fur, and body-weight curves were much better than in *Zmpste24*^{-/-}*Icmt*^{+/+} mice (Fig. 1, A to D, and fig. S4).

All *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice were sacrificed at 30 weeks of age, and none had rib fractures (Fig. 1, E and F). Osteoblasts in L2 vertebrae of *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice were six times as abundant as in *Zmpste24*^{-/-}*Icmt*^{+/+} mice; osteoclast numbers were unchanged (Fig. 1, G and H, and fig. S5B). The numbers, thickness, and bone volume of trabeculae were higher in *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice than in *Zmpste24*^{-/-}*Icmt*^{+/+} mice (fig. S5B). Moreover, at 13 to 15 weeks of age, *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice had increased mineral density and bone content (fig. S6A), as judged by dual x-ray absorptiometry (DXA). The DXA scans also revealed increased adipose tissue in *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice; however, total body weight at that time point did not differ (fig. S6B), consistent with the body-weight curves (Fig. 1B and fig. S4).

Misshapen nuclei are a hallmark of progeria cells. We initially suspected that the improved disease phenotypes of *Zmpste24*^{-/-}*Icmt*^{hm/hm} mice would be accompanied by a lower frequency of

misshapen nuclei in cultured fibroblasts, but this was not the case (Fig. 2, A and B). In control experiments, knockout of *Fntb* (encoding the FTase β-subunit) in *Zmpste24*^{-/-} fibroblasts reduced the frequency of misshapen nuclei to wild-type levels (fig. S7A).

We next determined whether reduced *Icmt* expression affects localization of prelamin A in *Zmpste24*^{-/-} cells. Consistent with earlier studies (17, 18), prelamin A was mainly found at the nuclear rim in *Zmpste24*^{-/-}*Icmt*^{+/+} hepatocytes, colocalizing with LAP2β (Fig. 2C). In contrast, prelamin A in *Zmpste24*^{-/-}*Icmt*^{hm/hm} hepatocytes was abundant in the nucleoplasm (Fig. 2C and fig. S7B). Similar results were observed in skeletal muscle (fig. S7C). Prelamin A, which accumulated at moderate levels in *Zmpste24*^{+/+}*Icmt*^{hm/hm} fibroblasts (fig. S2E), was entirely nucleoplasmic in liver sections (fig. S7D). The ratio of prelamin A and β-tubulin was similar in lysates of *Zmpste24*^{-/-}*Icmt*^{hm/hm} and *Zmpste24*^{-/-}*Icmt*^{+/+} tissues (fig. S7E). Thus, the hypomorphic *Icmt* allele partially mislocalizes prelamin A away from the nuclear rim but has no effect on the absolute levels of prelamin A.

Fibroblasts from *Zmpste24*^{-/-} mice proliferate slowly and undergo premature senescence (19, 20). We defined the impact of *Icmt* deficiency on those phenotypes. As expected,

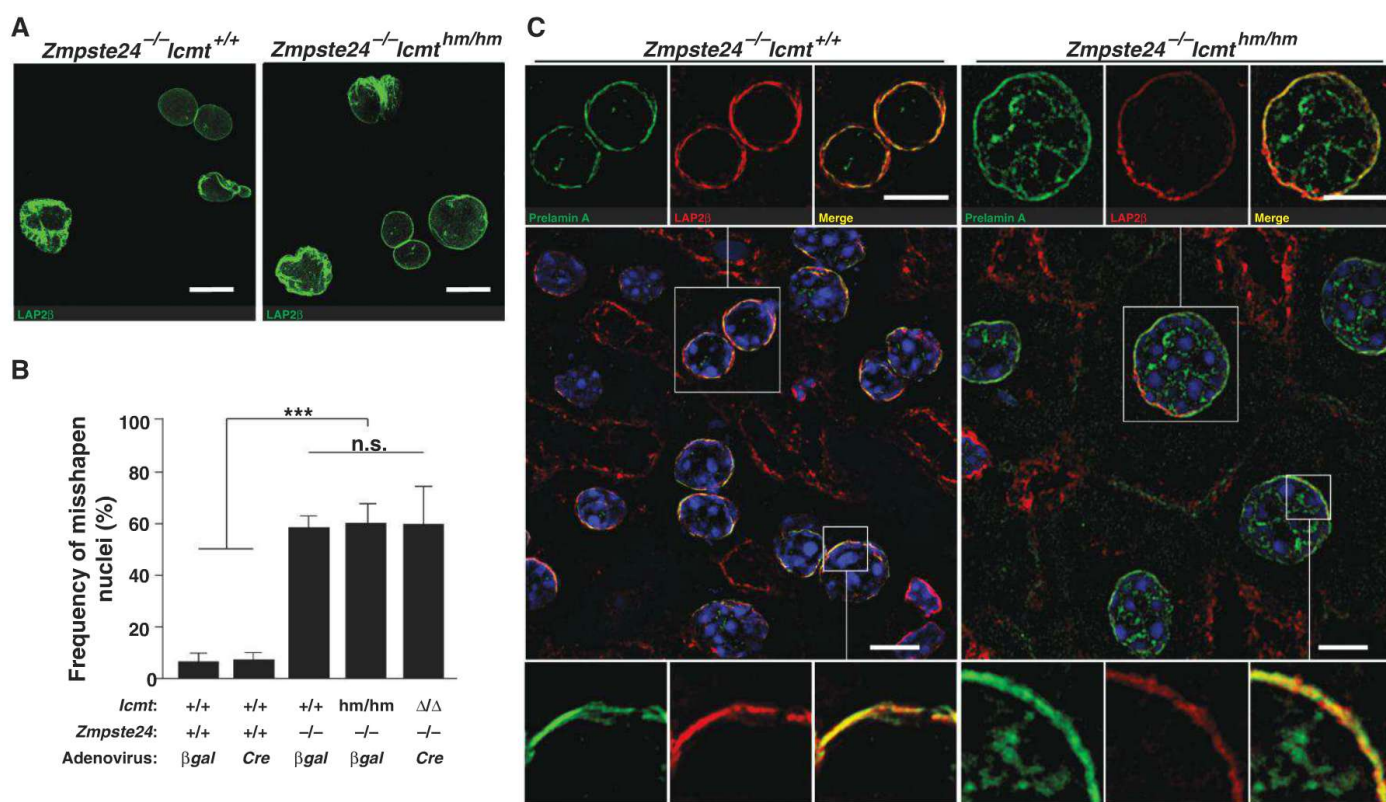


Fig. 2. *Icmt* deficiency mislocalizes prelamin A but does not reduce the frequency of misshapen nuclei in *Zmpste24*^{-/-} fibroblasts. (A) Confocal images of nuclei in primary mouse embryonic fibroblasts stained with a LAP2β antibody. Scale bar, 10 μm. (B) Frequency of misshapen nuclei in primary fibroblasts (*n* = 3 per genotype). Cre-adenovirus was used to produce

Zmpste24^{-/-}*Icmt*^{Δ/Δ} cells from the parental *Zmpste24*^{-/-}*Icmt*^{hm/hm} cells. βgal-adenovirus was used as control. (C) Super-resolution structured illumination microscopy (SR-SIM) immunofluorescence images of nuclei in liver sections stained with prelamin A- and LAP2β-specific antibodies and counterstained with 4',6-diamidino-2-phenylindole (DAPI). Scale bar, 10 μm. ****P* < 0.001.

Zmpste24^{-/-}*Icmt*^{+/+} fibroblasts proliferated slowly and senesced prematurely (Fig. 3A). In contrast, *Zmpste24*^{-/-}*Icmt*^{hm/hm} and *Zmpste24*^{-/-}*Icmt*^{ΔΔ} cells proliferated at rates similar to those of wild-type cells (Fig. 3A). Inactivating *Fntb* abolished cell proliferation (fig. S8).

We next explored the impact of *Icmt* deficiency on intracellular signaling pathways. The AKT-mTOR signaling pathway affects cell growth and survival (21). We observed higher levels of phosphorylated AKT and greater mTOR activation in *Zmpste24*^{-/-}*Icmt*^{hm/hm} cell lysates than in *Zmpste24*^{-/-}*Icmt*^{+/+} lysates, evident by increased phosphorylation of its downstream targets ribosomal protein S6 and 4E-BP1 (Fig. 3B). Similar results were observed in comparisons of *Zmpste24*^{+/+}*Icmt*^{hm/hm} and *Zmpste24*^{+/+}*Icmt*^{+/+} cells (fig. S9A). Consistent with increased rates of proliferation, *Zmpste24*^{-/-}*Icmt*^{hm/hm} cells had lower levels of the cyclin-dependent kinase

inhibitors p27^{KIP1} and p21^{CIP1} and the tumor suppressors p16^{INK4A} and phosphorylated retinoblastoma protein (Rb) (Fig. 3C). Levels of phospho-AKT and -S6 were lower in tissue lysates of *Zmpste24*^{-/-}*Icmt*^{+/+} mice than in *Zmpste24*^{+/+}*Icmt*^{+/+} mice; the levels were normalized in *Zmpste24*^{-/-}*Icmt*^{hm/hm} tissues (fig. S9, B to D). Cytosolic phospho-AKT can translocate into the nucleus and trigger inactivation and degradation of p21^{CIP1} (21). Levels of phospho-AKT were high in nuclei of *Zmpste24*^{-/-}*Icmt*^{hm/hm} hepatocytes and correlated with reduced levels of nuclear p21^{CIP1} (fig. S9E).

To determine whether increased AKT-mTOR signaling contributes to the increased proliferation of *Zmpste24*^{-/-}*Icmt*^{hm/hm} cells, we performed cell proliferation assays with inhibitors of AKT (GSK690693) and mTOR (rapamycin). The AKT inhibitor blocked the proliferation of *Zmpste24*^{-/-}*Icmt*^{hm/hm} cells, whereas the mTOR

inhibitor had no effect (Fig. 3D and fig. S10, A to C). We also activated AKT in *Zmpste24*^{-/-}*Icmt*^{+/+} cells with VO-OHPic, an inhibitor of phosphatase and tensin homolog (PTEN, a tumor suppressor upstream of AKT). VO-OHPic delayed the senescence of *Zmpste24*^{-/-}*Icmt*^{+/+} cells (Fig. 3E and fig. S10D). Thus, *Icmt* deficiency overcomes senescence of *Zmpste24*^{-/-} cells by activating AKT.

The increased AKT-mTOR signaling in *Icmt*-deficient cells was likely not caused by reduced methylation of RAS and RAS homologue enriched in the brain (RHEB) because their levels were unaffected by *Icmt* deficiency (fig. S11A). To test the possibility that the increased AKT-mTOR signaling was caused by unmethylated prelamins A, we analyzed fibroblasts from *Icmt*^{hm/hm} mice on a background of a mutant *Lmna* allele (*Lmna*^{LCO}) that produces lamin C but no prelamins A (22). The levels of phospho-AKT and -S6 were

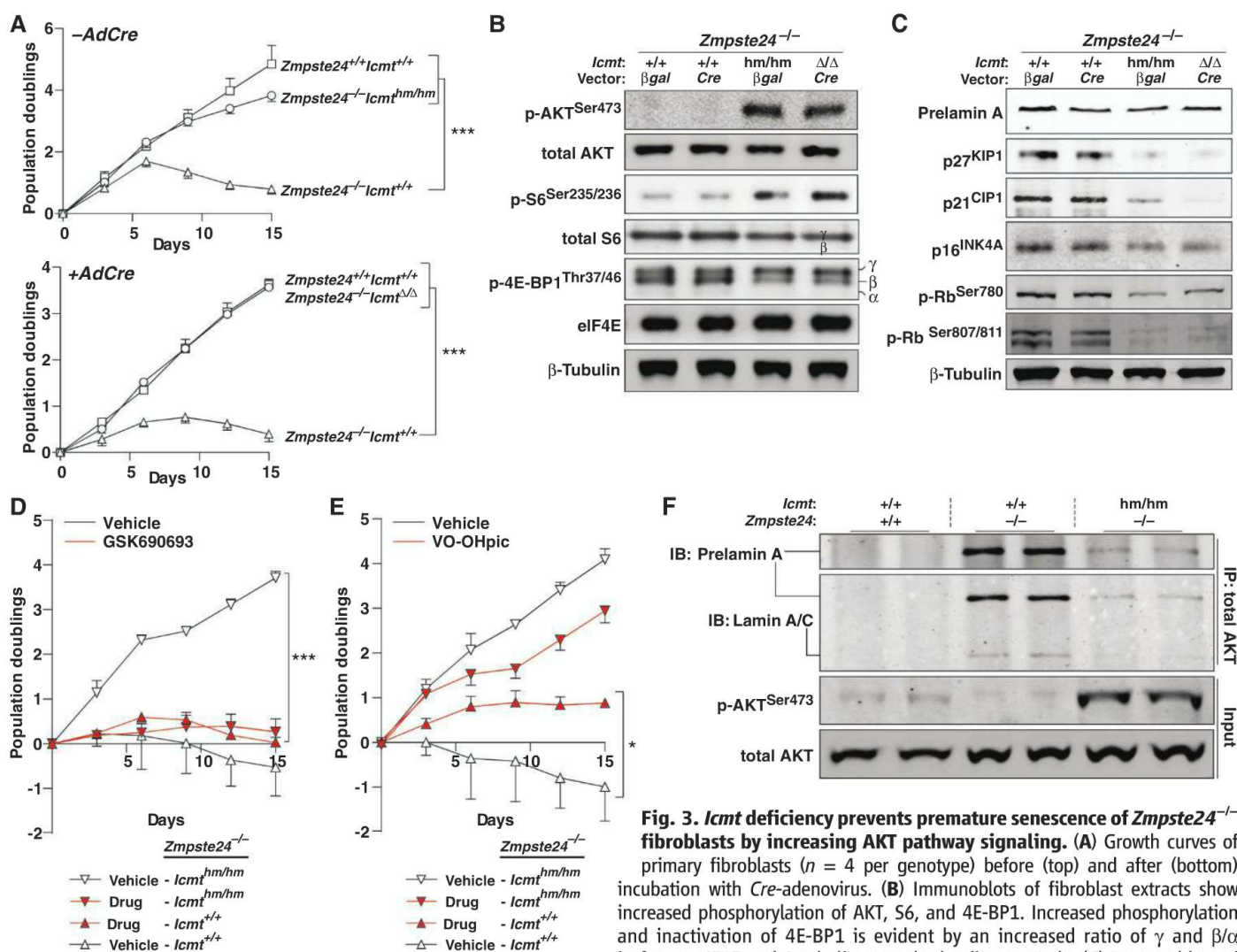


Fig. 3. *Icmt* deficiency prevents premature senescence of *Zmpste24*^{-/-} fibroblasts by increasing AKT pathway signaling. (A) Growth curves of primary fibroblasts (*n* = 4 per genotype) before (top) and after (bottom) incubation with Cre-adenovirus. (B) Immunoblots of fibroblast extracts show increased phosphorylation of AKT, S6, and 4E-BP1. Increased phosphorylation and inactivation of 4E-BP1 is evident by an increased ratio of γ and β/α isoforms. eIF4E and β-tubulin were the loading controls. (C) Immunoblots of

fibroblast extracts with antibodies against prelamins A and cell cycle regulatory proteins. (D and E) Growth curves of primary fibroblasts incubated with inhibitors of (D) AKT (GSK690693, 10 μM) and (E) PTEN (VO-OHPic, 150 nM) (*n* = 3/genotype). (F) Immunoprecipitation (IP) and immunoblot (IB) analyses showing a methylation-dependent association between AKT and prelamins A. The lysates were also used directly for immunoblots for AKT (input). **P* < 0.05; ****P* < 0.001.

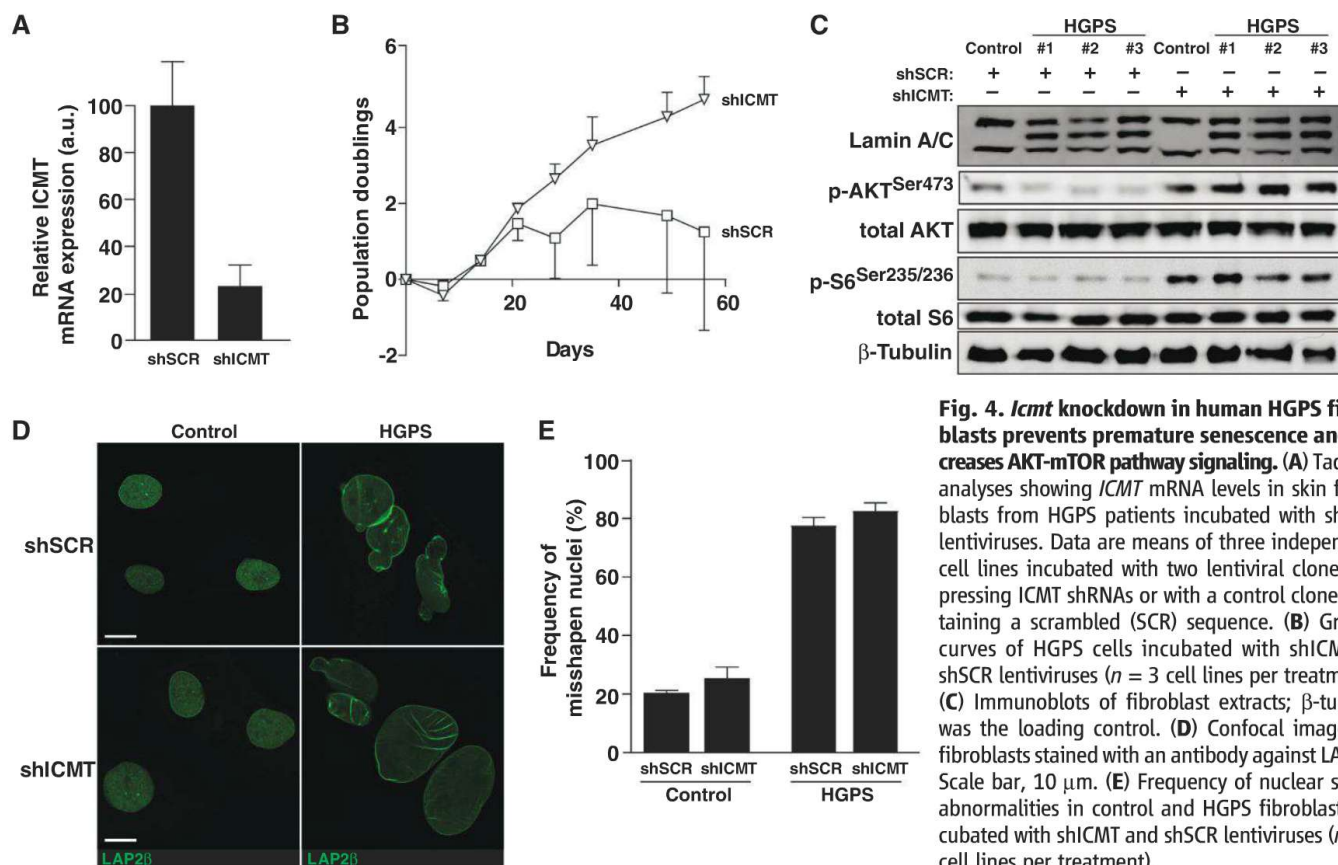


Fig. 4. *IcmT* knockdown in human HGPS fibroblasts prevents premature senescence and increases AKT-mTOR pathway signaling. (A) TaqMan analyses showing *ICMT* mRNA levels in skin fibroblasts from HGPS patients incubated with shRNA lentiviruses. Data are means of three independent cell lines incubated with two lentiviral clones expressing *ICMT* shRNAs or with a control clone containing a scrambled (SCR) sequence. (B) Growth curves of HGPS cells incubated with shICMT or shSCR lentiviruses ($n = 3$ cell lines per treatment). (C) Immunoblots of fibroblast extracts; β -tubulin was the loading control. (D) Confocal images of fibroblasts stained with an antibody against LAP2 β . Scale bar, 10 μ m. (E) Frequency of nuclear shape abnormalities in control and HGPS fibroblasts incubated with shICMT and shSCR lentiviruses ($n = 3$ cell lines per treatment).

lower in *IcmT*^{hm/hm}*Lmna*^{LCO/LCO} lysates than in *IcmT*^{hm/hm}*Lmna*^{+/+} lysates (fig. S11B). Thus, prelamin A is required for the increased AKT-mTOR signaling accompanying *IcmT* deficiency. Prelamin A and AKT were physically associated in *Zmpste24*^{-/-}*IcmT*^{+/+} lysates; the association was reduced in *Zmpste24*^{-/-}*IcmT*^{hm/hm} lysates (Fig. 3F). Perhaps an interaction between prelamin A and AKT in *Zmpste24*-deficient cells inhibits AKT-mTOR signaling (fig. S9, B to E).

To assess the effect of inhibiting ICMT in human fibroblasts, we suppressed ICMT expression in wild-type and HGPS fibroblasts with lentiviral short hairpin (sh) RNAs. The shRNAs reduced ICMT expression by 80% and had no discernible effect on the proliferation of fibroblasts from healthy subjects (Fig. 4A and fig. S12A). However, knockdown of ICMT in three HGPS cell lines delayed senescence and increased the mean proliferation rate (Fig. 4B and fig. S12B). The proliferation of HGPS cells also increased when ICMT activity was inhibited with *N*-acetyl-S-farnesyl-L-cysteine (AFC), a competitive ICMT inhibitor (fig. S12C). Rapamycin and an FTI dose-dependently reduced the proliferation of HGPS cells (fig. S12, D to G). The absolute levels of progerin were similar in shICMT-treated and control-HGPS cells, and phospho-AKT and -S6 levels were higher in shICMT-treated cells (Fig. 4C). Nuclear shape abnormalities in HGPS cells were unaffected by knockdown of ICMT

(Fig. 4, D and E). Thus, cells from HGPS patients and *Zmpste24*-deficient mice respond similarly to ICMT inhibition.

Our findings raise the possibility that targeting ICMT could be an effective strategy for treating HGPS. Reduced *IcmT* expression was accompanied by improved disease phenotypes in *Zmpste24*^{-/-} mice. Thus, the favorable effects in the setting of a hypomorphic *IcmT* allele suggest that the efficacy of ICMT inhibitors would not require complete inhibition of the enzyme.

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Supplementary Materials

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Materials and Methods
Figs. S1 to S12
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The Molecular Basis for Attractive Salt-Taste Coding in *Drosophila*

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Below a certain level, table salt (NaCl) is beneficial for animals, whereas excessive salt is harmful. However, it remains unclear how low- and high-salt taste perceptions are differentially encoded. We identified a salt-taste coding mechanism in *Drosophila melanogaster*. Flies use distinct types of gustatory receptor neurons (GRNs) to respond to different concentrations of salt. We demonstrated that a member of the newly discovered ionotropic glutamate receptor (IR) family, IR76b, functioned in the detection of low salt and was a Na⁺ channel. The loss of IR76b selectively impaired the attractive pathway, leaving salt-aversive GRNs unaffected. Consequently, low salt became aversive. Our work demonstrated that the opposing behavioral responses to low and high salt were determined largely by an elegant bimodal switch system operating in GRNs.

To address the fundamental question of how low- and high-salt taste perceptions are differentially encoded in gustatory receptor neurons (GRNs) in insects, we chose the fruit fly as a model. We first tested the animal's behavioral responses to different salt concentra-

tions ranging from 1 to 1000 mM, using a robust, food-color-based preference assay (fig. S1, A and B). Akin to mammals, flies preferred low-salt food (1 to 100 mM), with a maximal preference at 50 mM NaCl, whereas they rejected high-salt food (≥ 200 mM) (Fig. 1A) (1–3). This pattern

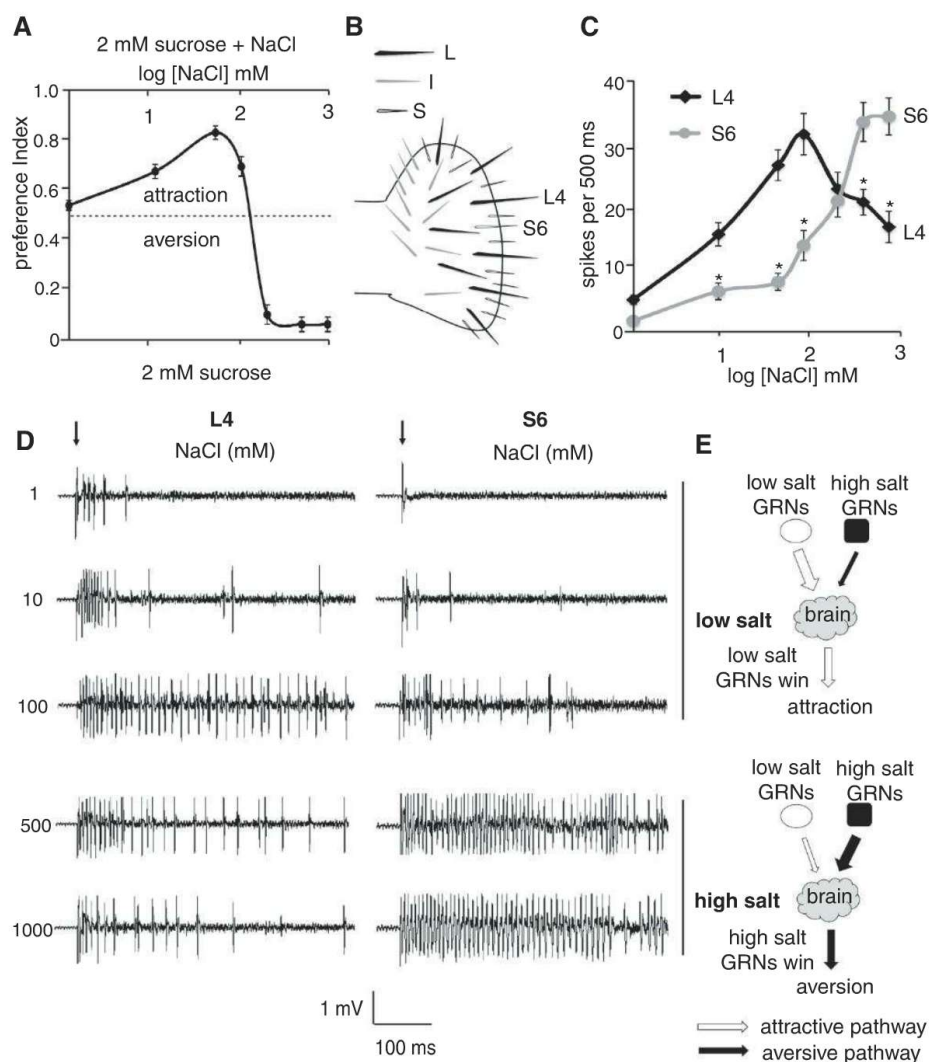
differs from both sugar and bitter taste in that flies prefer sweet and dislike bitter compounds regardless of the concentration.

In *Drosophila*, the primary taste sensory organ, the labelum, contains 31 sensilla, which are further classified by size as small (S), intermediate (I), and large (L) sensilla (Fig. 1B) (4). Sensilla contain multiple GRNs, which respond to distinct stimuli, including bitter, sweet, and salty tastants (4–6). We surveyed the physiological responses of sensilla to low salt (50 mM) and high salt (500 mM) by performing tip recordings (7) (fig. S2). The GRNs housed by two L-type sensilla (L4 and L6) produced the most robust firings in response to low salt, whereas the GRNs in three S-type sensilla (S4, S6, and S8) displayed the strongest responses to high salt (fig. S2, A

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Fig. 1. Wild-type responses to different concentrations of salt. (A) Behavioral responses to 1 to 1000 mM salt. The dashed line indicates no preference. $n = 10$ trials with ~70 flies per trial. (B) Cartoon showing the distribution of L-, I-, and S-type sensilla in the labelum. (C and D) Tip recordings using L4 and S6 sensilla in response to low salt. $n = 10$. The arrows indicate application of the recording electrode to the sensilla. Error bars indicate SEMs; $*P < 0.01$. (E) Schematic depicting how the L- and S-type sensilla differentially mediated attractive and aversive salt taste.



and C). These three S-type sensilla respond differentially to bitter tastants (8), suggesting that each type of gustatory sensillum has a unique taste tuning profile. GRNs in I-type sensilla responded to salt (9), but none as robustly as the most sensitive L- or S-type sensilla (fig. S2). With the exception of S4 and S8, the S-type sensilla show robust responses to the broadest array of aversive tastants (5, 8), whereas L-type sensilla produce the strongest physiological responses to attractive tastants, such as sugars (6, 8, 10, 11). Thus, we deduced that the responses to low and high salt (5, 6, 12) were likely to be controlled by a balance between the GRNs housed in L- and S-type sensilla.

We focused on L4 and S6 sensilla, using NaCl concentrations ranging from 1 to 1000 mM (Fig. 1, C and D). The firing of salt GRNs in the L4 sensilla increased progressively at low concentrations and peaked at 100 mM. In contrast, the salt GRNs in S6 sensilla were much less active than the salt GRNs in L4 sensilla, suggesting that these latter sensilla played a predominant role in low salt response. As the salt concentration increased above 100 mM, the firing of salt GRNs in L4 sensilla gradually declined. In contrast, the action potentials produced by S6 salt GRNs exhibited a remarkable increase (≥ 100 mM), with a maximal response at 500 mM. At high salt

concentrations, the firing of S6 salt GRNs far exceeded that of L4 salt GRNs, indicating that the high-salt response was controlled predominantly by salt GRNs in S-type sensilla.

We therefore propose a model in which competition between GRNs in the S- and L-type sensilla accounts for the bidirectional behavioral responses to salt. At low concentrations, the low-salt GRNs dominate over the high-salt GRNs, thereby causing the animals to prefer low salt (Fig. 1E). At high salt levels, the high-salt GRNs overwhelm the low-salt GRNs, resulting in salt rejection.

We next tested several candidate salt receptors and channels, none of which affected salt taste (supplementary text). Ionotropic receptors (IRs) are a class of olfactory receptors in *Drosophila* that are distantly related to mammalian ionotropic glutamate receptors (iGluRs) (13). Several *Ir* genes, such as *Ir25a* and *Ir76b*, also appear to be expressed in gustatory sensilla (13–15). The *Ir25a*² mutant had no obvious deficits in sensing either low salt or high salt (fig S3, A and C). We then carried out a genetic analysis of *Ir76b* and generated two null alleles, *Ir76b*¹ and *Ir76b*², by P-element-mediated imprecise excision (Fig. 2A). We also retained a revertant line that underwent a precise P-element excision (*Ir76b*^{R1}). Loss of *Ir76b* did not impair the re-

sponses to potassium chloride, sucrose, water, or bitter tastants (fig. S4, A to C).

The *Ir76b* deletions resulted in severe defects in the attraction to low salt concentrations (1 to 100 mM; Fig. 2B). In contrast, the *Ir76b* mutants showed the same aversion to high salt as the *Ir76b*^{R1} control (*w*¹¹¹⁸). *Ir76b*^{R1} behavior was indistinguishable from the wild-type *Ir76b*⁺ control (*w*¹¹¹⁸) at all salt concentrations (Fig. 2B).

We next performed tip recordings to monitor physiological abnormalities in the GRNs. The *Ir76b* mutations caused a decrease in the number of action potentials by salt GRNs in L4 sensilla in response to 50 mM salt (Fig. 2, C and D), demonstrating a functional defect in the GRNs. There were no significant changes in the firing frequencies of S6 GRNs in the *Ir76b* mutants, as compared with the wild type (Fig. 2, C and D). Using the *Ir76b-Gal4* and *UAS-Ir76b* transgenes, we restored normal attractive responses to low salt in the *Ir76b*¹ mutant (Fig. 2, C to E).

We also examined the physiological responses to different salt concentrations (1 to 1000 mM NaCl). Loss of *Ir76b* caused significant reductions in the firing frequencies of the salt GRNs in L4 sensilla at all salt concentrations (Fig. 2E). The firing of salt GRNs in L6 sensilla was also impaired (fig. S4D). However, there were no effects on the physiological responses of

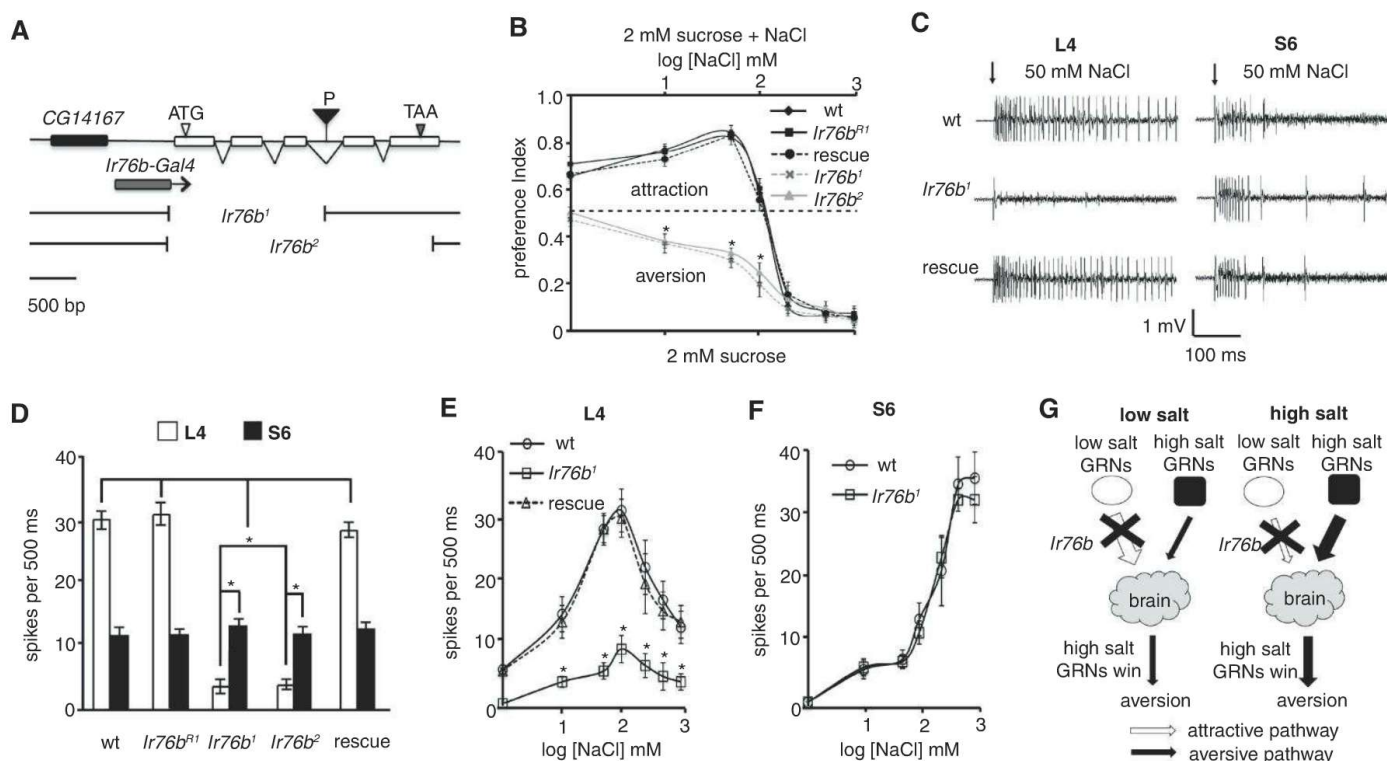


Fig. 2. *Ir76b* is required for low-salt preference. (A) Genomic organization of *Ir76b*. Shown are a P element (P) inserted in *Ir76b*, the deletions in *Ir76b*¹ and *Ir76b*², and the genomic region included in the *Ir76b-Gal4* transgene. (B) Two-way choice tests of sucrose versus sucrose plus salt. The rescue flies were *Ir76b*^{R1} flies harboring the *UAS-Ir76b* and the *Ir76b-Gal4* transgenes. *n* = 10. (C and D)

50 mM NaCl-induced action potentials in L4 and S6 sensilla. *n* = 15. (E and F) Action potential frequencies produced by L4 (*n* = 5) and S6 (*n* = 3) sensilla across different NaCl concentrations. Analysis of variance tests were performed. (G) Cartoon showing that the loss of *Ir76b* selectively disrupted the attractive salt taste pathway. Error bars indicate SEMs; **P* < 0.01.

high-salt GRNs in S4 or S6 sensilla (Fig. 2F and fig. S4E).

Taken together, our studies indicated that the removal of *Ir76b* selectively disrupted the attractive salt pathway, while leaving the aversive salt pathway intact (Fig. 2G). Consequently, *Ir76b* mutant animals avoided rather than preferred low salt, whereas they retained aversion to high salt.

To examine the cellular distribution pattern of IR76b, we raised antibodies against IR76b. The antibodies marked GRNs in the labelum, and the staining was virtually eliminated in *Ir76b* mutants (Fig. 3, A and B, and fig. S5A). We also generated flies expressing an *Ir76b* reporter

(*Ir76b-Gal4*). In combination with *UAS-mCD8::GFP* or *UAS-dsRed*, we detected prominent GRN staining in the proboscis (Fig. 3, C and D). *Ir76b*-expressing GRNs were housed in all L-type sensilla, including L4 and L6 (Fig. 3C). We also detected *Ir76b* reporter expression in GRNs in the leg tarsi and wing margins, which sent projections to the ventral nerve cord (fig. S5, E to G). *Ir76b* reporter expression largely overlapped with the anti-IR76b staining, suggesting that the reporter reflected the bona fide cellular distribution of *Ir76b* (fig. S5, B to D).

To determine whether *Ir76b*-positive GRNs overlapped with *Gr66a*-expressing bitter-responsive

or *Gr5a*-expressing sugar-responsive GRNs, we generated an *Ir76b* reporter using the Q system (*Ir76b-QF*) (16). Double labeling showed that *Ir76b*-positive GRNs were distinct from either *Gr66a* or *Gr5a* GRNs (Fig. 3, E and F). *Gr66a*- and *Gr5a*-positive GRNs project their axons from the labelum to non-overlapping portions of the subesophageal ganglion (SOG) in the brain (17, 18). The projections of *Ir76b* GRNs in the SOG (Fig. 3G and fig. S5H) showed minimal overlap with regions innervated by the axons of *Gr5a* and *Gr66a* GRNs (Fig. 3, H and I). Thus, *Ir76b* GRNs represented a class of GRNs distinct from sugar- or bitter-responsive GRNs.

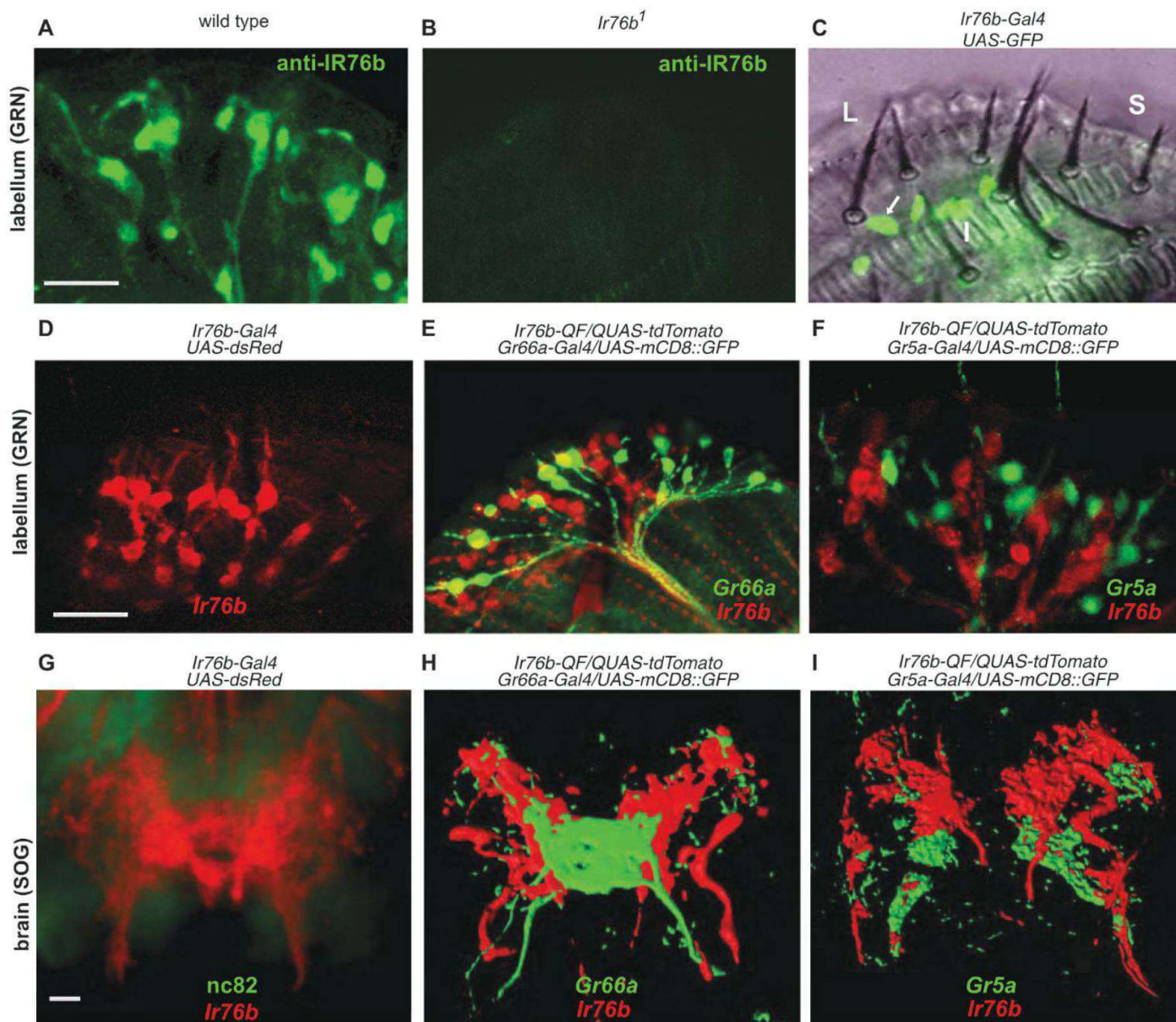


Fig. 3. Expression of *Ir76b* in GRNs. (A and B) Anti-IR76b staining of wild-type and *Ir76b*¹ labela. (C) Green fluorescent protein (GFP) fluorescence superimposed on a bright-field view of a *Ir76b-Gal4/UAS-mCD8::GFP* labelum. The arrow indicates a GFP-positive GRN within an L4 sensillum. (D) *Ir76b* reporter staining a labelum. (E) Labelum expressing *Ir76b* (red) and *Gr66a*

(green) reporters. (F) Labelum expressing *Ir76b* (red) and *Gr5a* (green) reporters. (G) Projections of GRNs in the SOG expressing an *Ir76b* reporter. (H and I) Three-dimensional reconstructions of the GRN projections in the SOG, using flies expressing *Ir76b* (red), *Gr66a* (green), and *Gr5a* (green) reporters as indicated. Scale bars, 10 μm.

In olfactory receptor neurons (ORNs), IRs function either alone or in conjunction with other IRs (13, 19). We tested whether misexpression of IR76b alone conferred salt taste when introduced in non-salt-responsive GRNs. Because *Ir76b* and *Gr5a* are expressed in different GRN populations, we introduced *Ir76b* in *Gr5a*-sugar neurons in an *Ir76b*¹ background. We recorded from L2 sensilla, which showed few responses to low salt even in the wild type (Fig. 4A). In response to NaCl, there was a robust train of action potentials produced by these *Gr5a* GRNs in L2 sensilla (Fig. 4, A and B, and fig. S6A). In contrast, these same GRNs did not induce a response to NMDGCl or potentiate the response to sucrose (Fig. 4, A and B). Thus, the action potentials were due to Na⁺ and not Cl⁻ and were not a consequence of nonspecific elevation of *Gr5a* GRN activity. The behavioral deficit in low-salt preference in *Ir76b* mutants was rescued by misexpressing *Ir76b* in *Gr5a* GRNs (Fig. 4C).

To test whether IR76b was capable of functioning as a Na⁺-permeable channel, we performed whole-cell recordings after expressing IR76b in HEK293T cells (fig. S7, A and B). The IR76b-expressing cells showed increased current (*I*_{IR76b}) relative to control cells (Fig. 4D). The nearly linear current-voltage (*I*-*V*) relationship indicated that *I*_{IR76b} was not strongly voltage-dependent

(Fig. 4E). Replacement of the external Cl⁻ with gluconate anions had little effect on *I*_{IR76b} (fig. S7D). Using bionic conditions, the relative ion selectivity of IR76b was $P_{Na}(1.0) = P_{Cs}(1.0) > P_K(0.4)$ (Fig. 4E and fig. S7, C and E). The Na⁺ conductance properties of IR76b were similar to those of NALCN, a mouse Na⁺ leak channel (20), and suggested that IR76b was in a constitutively open state.

The ion conductance of iGluRs is controlled by residues in the third transmembrane (TM3) region that includes YTANLAAFLT (21). In the absence of ligand, the channels are closed. A spontaneous A288T mutation in TM3 of mouse GluR82 (Lurcher mutation; GluR82^{Lc}) disrupts the closed conformation, resulting in a constitutive Na⁺ conductance (22). IR76b harbored a threonine (T293) in nearly the same position as the Lurcher substitution (A288T; Fig. 4F and fig. S7, F and G). A threonine is absent in corresponding positions of other fly IRs and mammalian iGluRs (Fig. 4F). Therefore, we postulated that T293 enabled IR76b to be fixed in an open Na⁺-permeable state. To test this idea, we replaced IR76b with IR76b^{T293A} and determined the effects of this substitution in vivo and in vitro. When expressing *UAS-Ir76b*^{T293A} using *Gr5a-Gal4*, we did not detect a salt response in L2 sensilla (Fig. 4A). Moreover, the T293A mutation greatly

attenuated the constitutive current in HEK293T cells (Fig. 4, D and E).

To explain how the fly uses IR76b to detect salt, we propose that IR76b is a Na⁺ leak channel and is effective because of the unusual extracellular cation composition bathing the GRNs. Different from the body hemolymph, which contains high Na⁺, the endolymph that bathes insect chemosensory neurons appears to have a low Na⁺ concentration (23). Under resting conditions, there may be little Na⁺ conductance. After consuming Na⁺-containing foods, the Na⁺ levels in the endolymph rise, driving Na⁺ influx through IR76b. The excitation of salt-attractive GRNs induces the animals to consume salt. Loss of IR76b selectively impaired the attractive pathway, making the otherwise attractive low salt become aversive.

Our work establishes that the salt attractive pathway relies on a type of Na⁺-permeable channel not previously known to function in taste, and this channel, IR76b, bears no relationship to epithelial sodium channels (ENaCs) that are required for sensing low salt in mice (24). Some ENaC channels may be constitutively active (25) and lead to the depolarization of taste receptor cells after a rise in cation levels at the cell surface (26). Thus, despite the divergence between fly IRs and mammalian ENaC channels, they may mediate salt taste through similar mech-

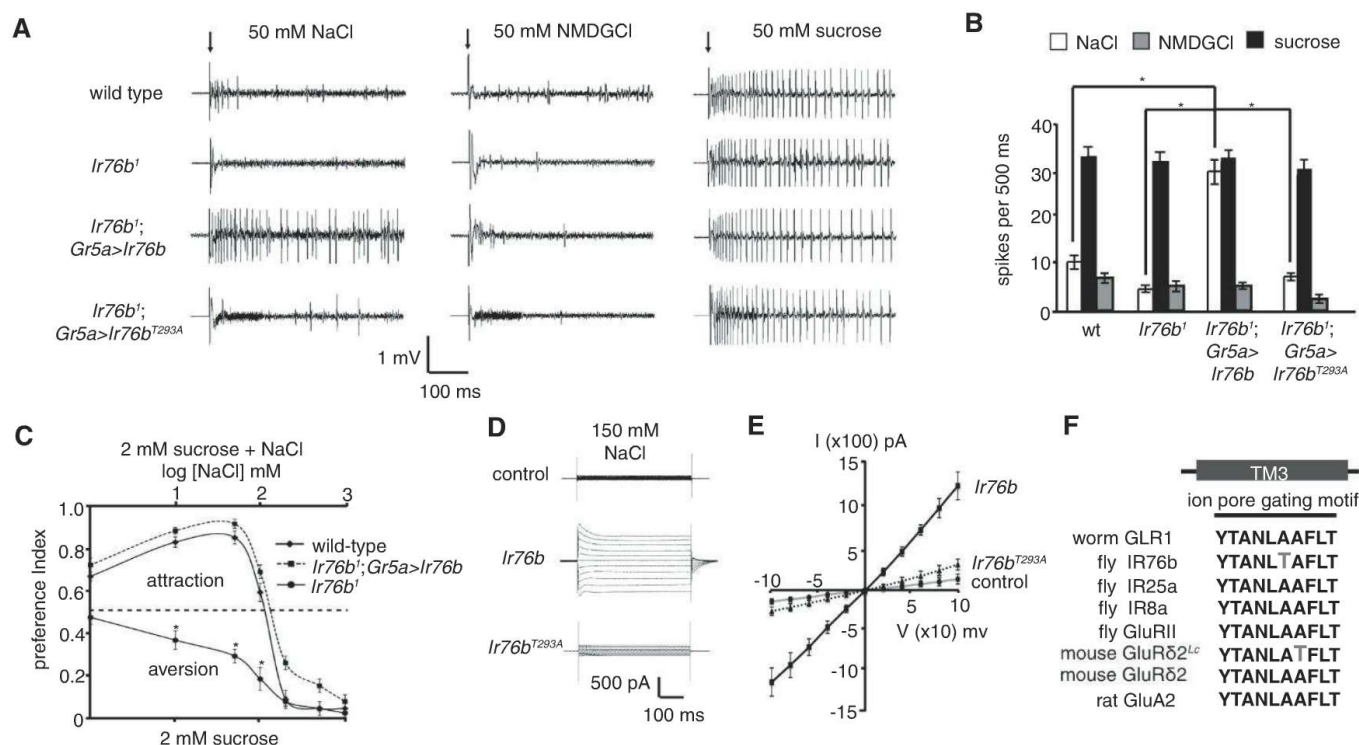


Fig. 4. *Ir76b* was sufficient to function as a salt sensor. (A and B) Tip recordings and quantification showing action potentials triggered by 50 mM NaCl after misexpression of *Ir76b* in *Gr5a* GRNs. NMDGCl and sucrose were negative and positive controls, respectively. $n = 10$. (C) Two-way choice tests (2 mM sucrose versus 2 mM sucrose plus different concentrations of NaCl) after misexpression of *UAS-Ir76b* using *Gr5a-Gal4*. $n = 5$. (D) Whole-cell

voltage clamp recordings of HEK293T cells expressing wild-type IR76b or IR76b^{T293A} (with 150 mM NaCl in the bath). The cells were stimulated with voltage steps of 500 ms duration (−100 mV to +100 mV in 10-mV increments). (E) *I*-*V* relationships of cells expressing IR76b or IR76b^{T293A} (with 150 mM NaCl in the bath). Error bars indicate SEMs; * $P < 0.01$. (F) Sequences of the ion pore gating motif in TM3 of the indicated iGluRs and IRs.

animals. Our competition model for low- and high-salt taste detection may represent a widely used mechanism for salt-taste coding in other animals, including mammals.

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Supplementary Materials

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Materials and Methods

Supplementary Text

Figs. S1 to S7

References

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Parallel Neural Pathways Mediate CO₂ Avoidance Responses in *Drosophila*

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Different stimulus intensities elicit distinct perceptions, implying that input signals are either conveyed through an overlapping but distinct subpopulation of sensory neurons or channeled into divergent brain circuits according to intensity. In *Drosophila*, carbon dioxide (CO₂) is detected by a single type of olfactory sensory neuron, but information is conveyed to higher brain centers through second-order projection neurons (PNs). Two distinct pathways, PN_v-1 and PN_v-2, are necessary and sufficient for avoidance responses to low and high CO₂ concentrations, respectively. Whereas low concentrations activate PN_v-1, high concentrations activate both PN_vs and GABAergic PN_v-3, which may inhibit PN_v-1 pathway-mediated avoidance behavior. Channeling a sensory input into distinct neural pathways allows the perception of an odor to be further modulated by both stimulus intensity and context.

Insects detect odor with olfactory sensory neurons (OSNs), which converge to the antennal lobe (AL) before conveyance to the mushroom body (MB) and lateral horn (LH) via stereotyped projection neurons (PNs) (1–4). In *Drosophila*, carbon dioxide (CO₂) concentrations lower than 2% activate only one type of OSN that expresses *Gr21a* and *Gr63a* receptors and projects to a single V-glomerulus (5–9). We examined the morphology and functionality of PNs innervating the V-glomerulus (PN_vs) with regard to CO₂ responses.

We expressed a photoactivatable green fluorescent protein (PaGFP) in ~60% of neurons

using *Cha-Gal4>UAS-PaGFP* flies (10–12) and labeled candidate PN_vs by means of targeted photoconversion (fig. S1A). Although nearby tracts could have been labeled, the circuits were complemented and validated by browsing single neuron representations in the FlyCircuit database (13). Up to 12 heteromorphic PN_vs with different morphologies may link the V-glomerulus and higher brain centers (figs. S1B and S2) via the inner, medial, and outer antennocerebral tracts (iACT, mACT, and oACT, respectively).

To assess the functional roles of these PN_vs, we identified 50 *Gal4* lines that labeled the V-glomerulus, including seven putative PN_vs. We used genetic mosaic analyses to resolve individual neurons, using either repressible cell marker (MARCM) (14), FLP-out (2), or *Brainbow* (fig. S3) techniques to identify four genetically addressable PN_vs: PN_v-1 (*VT33008-Gal4*, *VT1606-Gal4*, *VT31497-Gal4*, and *VT48643-Gal4*) (Fig. 1, A to D), which links the bilateral V-glomeruli via oACT to the lateral horn (LH) and calyx (Cal); PN_v-2 (*E0044-Gal4*) (Fig. 1E), which

connects a single V-glomerulus via iACT to the bilateral superior dorsofrontal protocerebrum (SDFP); PN_v-3 (*VT12760-Gal4*) (Fig. 1F), which innervates all glomeruli of a single AL and projects via mACT to the LH, inner dorso-lateral protocerebrum (IDL), and SDFP; and PN_v-4 (*E0564-Gal4*) (Fig. 1G), which links two ALs via oACT to the SDFP, superpeduncular protocerebrum (SPP), caudal ventrolateral protocerebrum (CVLP), IDLP, and LH. Their termini are primarily localized to the SDFP and LH (fig. S4) (13).

Using *Dscam*[*exon17.1*]:GFP as a dendritic marker (15), we demonstrated that they all project into the V-glomerulus (Fig. 1, Ab–Gb). We used the GRASP (green fluorescent protein reconstitution across synaptic partners) technique to assess whether these dendrites receive input from CO₂ OSNs (16, 17). Half of the split-GFP GRASP reporter was expressed in OSN_v neurons by using *L5131-LexA* (fig. S5Aa), which specifically labels OSNs (fig. S5Ab) that are *Gr21a-nlsDsRed*-positive (fig. S5B), innervate the V-glomerulus (fig. S5Ca), and respond to 0.5% CO₂ (fig. S5C, b and c). The other half was expressed in distinct PN_vs by using an appropriate *Gal4* driver. In all cases, GRASP signals were observed in the V-glomerulus (Fig. 1, Ac to Gc). In the control experiment using *Mz19-Gal4* expressed in PNs innervating several other glomeruli (fig. S5D), GRASP signals were absent in the V-glomerulus (fig. S5E).

Monitoring functional responses in the V-glomerulus with a genetically encoded calcium indicator (GCaMP) revealed that all four PN_v types responded to CO₂ but not air, methylcyclohexanol (MCH), or octanol (OCT). PN_v-1 and PN_v-4 responded equally to 0.5 and 2% CO₂, whereas PN_v-2 and PN_v-3 responded dose-dependently (Fig. 1, Ae to Ge). Quantitative fluorescence measurement showed that basal GCaMP expression driven by seven *PN_v-Gal4* lines varied more than twofold (fig. S6A). A functional curve to different CO₂ concentrations showed that CO₂-

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response kinetics were independent of Gal4 efficiency (fig. S6B). Although GCaMP imaging is not fast enough to monitor temporal PN responses (18), the degrees of GCaMP change

were always $PN_V-1 > PN_V-2 > PN_V-4 > PN_V-3$ (fig. S6B). PN_V-1 has significantly more dendrites than other PN_V s (Fig. 1, Ad to Gd, and fig. S7). PN_V-1 and PN_V-2 only accounted for

one neuron in each hemisphere (Fig. 1 and fig. S16). The three isomorphic PN_V-3 s exhibited similar CO_2 -response kinetics (fig. S8), which is consistent with direct intracellular recording showing that DM6 PNs with similar morphologies innervating the same glomerulus exhibit similar odor-response properties (19).

Are any of these CO_2 -responsive PN_V s necessary for avoidance behavior? Avoidance to 0.5% CO_2 was impaired when PN_V-1 neurotransmission was acutely disrupted with $UAS-shi^{ts1}$ at 30°C (Fig. 2A) but not 21°C (Fig. 2B). These flies exhibited normal avoidance to MCH and OCT at 30°C (fig. S9), and 0.5% CO_2 avoidance was normal when PN_V-2 , PN_V-3 , or PN_V-4 neurotransmission was disrupted at 30°C (Fig. 2A). A different pattern emerged for 2% CO_2 . Avoidance behavior was normal when neurotransmission from PN_V-1 , PN_V-3 , or PN_V-4 was blocked (Fig. 2C). Instead, 2% CO_2 avoidance was impaired when PN_V-2 neurotransmission was disrupted with $UAS-shi^{ts1}$ at 30°C (Fig. 2C) but not 21°C (Fig. 2D). We verified this by silencing neural activity with adult-stage-specific expression of inwardly rectifying potassium channel Kir2.1 (20). Silencing PN_V-1 only impaired 0.5% CO_2 avoidance, and silencing PN_V-2 only impaired 2% CO_2 avoidance. Silencing PN_V-3 or PN_V-4 did not affect CO_2 avoidance (fig. S10).

For PN_V-2 , we identified $E0044-Gal4$ after screening thousands of Gal4 expression patterns, including those in FlyLight (21) and BrainBase (<http://brainbase.imp.ac.at>). Though $E0044-Gal4$ expression is specific in the brain (Fig. 1Ea), it is important to confirm that the observed changes are not due to other neuronal types. $E0044-Gal4$ was also expressed in paired dorsal anterior lateral (DAL) neurons, a few ascending subesophageal ganglion neurons, several descending pars intercerebralis neurons, some sensory input neurons terminating in the ALs and antennal mechanosensory motor center (AMMC), and brain-surface glial cells (fig. S11A). First, 2% CO_2 avoidance was impaired by blocking neurotransmission with $UAS-shi^{ts1}$ from $E0044-Gal4$ (fig. S12A) but normalized when Gal4 expression was inhibited by $Cha-Gal80$ (fig. S12B), indicating that Cha^+ neurons are involved. Second, impaired 2% CO_2 avoidance was unaffected by blocking PN_V-2 neurotransmission in flies carrying $ey-flp/E0044-Gal4;UAS>shi^{ts1}>stop$; $cry-Gal80$ transgenes that exclude DAL and sensory neurons (fig. S11, B and C) (22). Third, blocking neurotransmission in DAL neurons with specific and strong $G0431-Gal4$ driver did not impair 2% CO_2 avoidance (fig. S11, D to H). Last, GCaMP responses to 2% CO_2 were only evident in PN_V-2 s in the V-glomerulus (dendrites) and SDFP (axons) (fig. S11I).

Would activation of either PN_V-1 or PN_V-2 alone with a blue light-gated ion channel channelrhodopsin-2 (ChR2) (23, 24) be sufficient to elicit CO_2 avoidance behavior? We used a modified optogenetic T-maze (8) (fig. S13A) equipped with blue and yellow light-emitting diodes (LEDs) (fig. S13B)

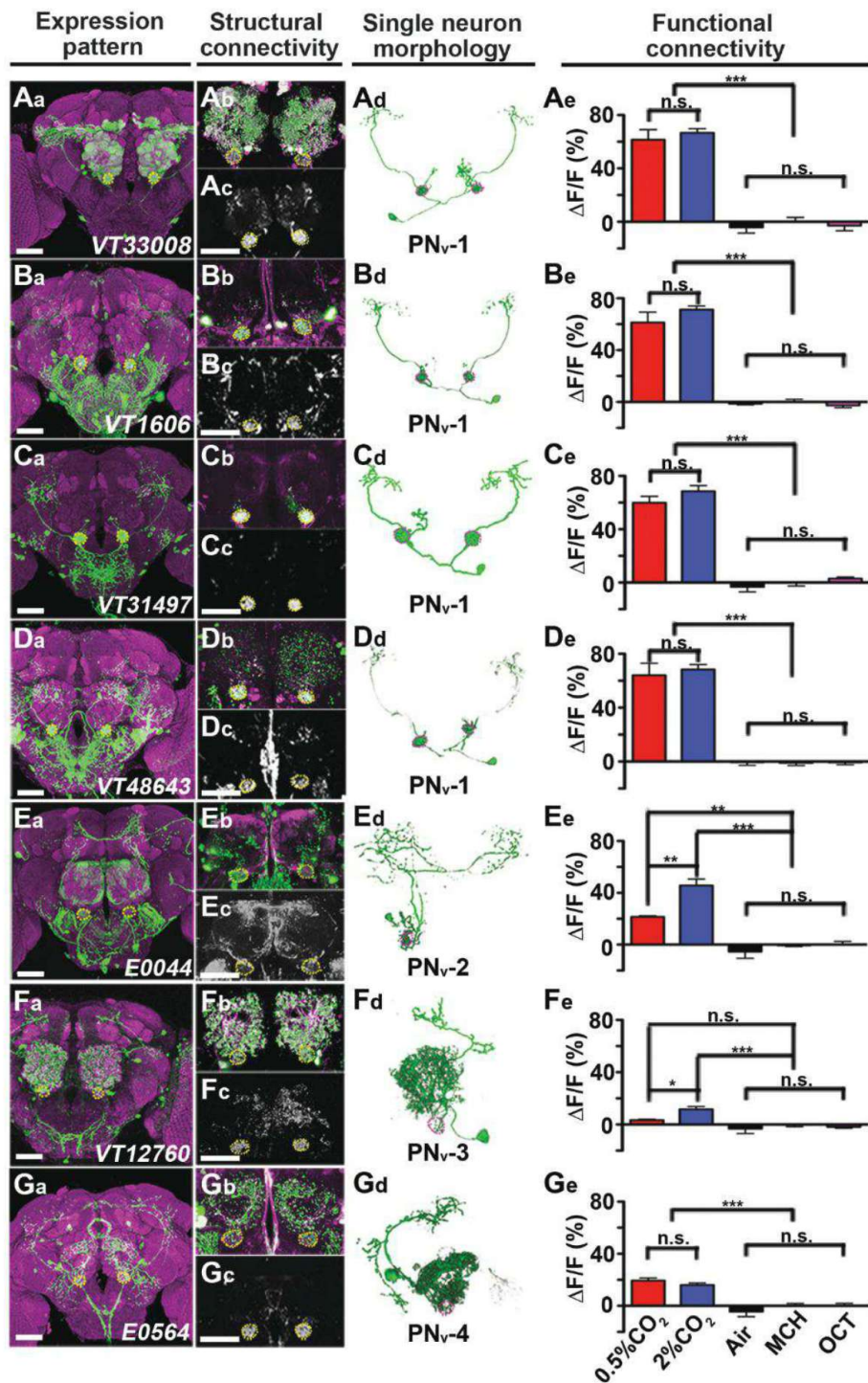


Fig. 1. PN_V s structural and functional connectivity. (Aa to Ga) Expression patterns of seven different PN_V-Gal4 s with V-glomerulus (dotted circle) innervations. $Gal4$ neurons were labeled with $UAS-mCD8::GFP$ (green), and brain neuropils were immunostained with a DLG antibody (magenta). (Ab to Gb) Putative dendrites in the V-glomerulus labeled with $UAS-Dscam[17.1]::GFP$ (green). $Gal4$ neurons were labeled with $UAS-mKO$ (magenta). (Ac to Gc) GRASP visualization of structural contacts between $L5131-LexA$ and PN_V-Gal4 s. Scale bar, 50 μm . (Ad to Gd) Morphology of a single PN_V . (Ae to Ge) GCaMP changes ($\Delta F/F_0$) in the V-glomerulus in response to odor stimuli. Each value is mean $\pm$ SEM ($n = 6$ to 10 samples, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). More detailed legends of this and the other figures are available in the supplementary materials.

Fig. 2. PN_v-1 and PN_v-2 outputs are necessary to elicit avoidance to 0.5 and 2% CO₂, respectively. (A and B) Avoidance response to 0.5% CO₂ in PN_v-Gal4>UAS-shi^{ts1} flies at 30°C (A) and 21°C (B). (C and D) Avoidance response to 2% CO₂ in PN_v-Gal4>UAS-shi^{ts1} flies at 30°C (C) and 21°C (D). Seven different PN_v-Gal4 drivers were used: PN_v-1a (VT33008-Gal4), PN_v-1b (VT1606-Gal4), PN_v-1c (VT31497-Gal4), PN_v-1d (VT48643-Gal4), PN_v-2 (E0044-Gal4), PN_v-3 (VT12760-Gal4), and PN_v-4 (E0564-Gal4). Each value is mean ± SEM (n = 6 to 8 experiments, **P < 0.01, ***P < 0.001).

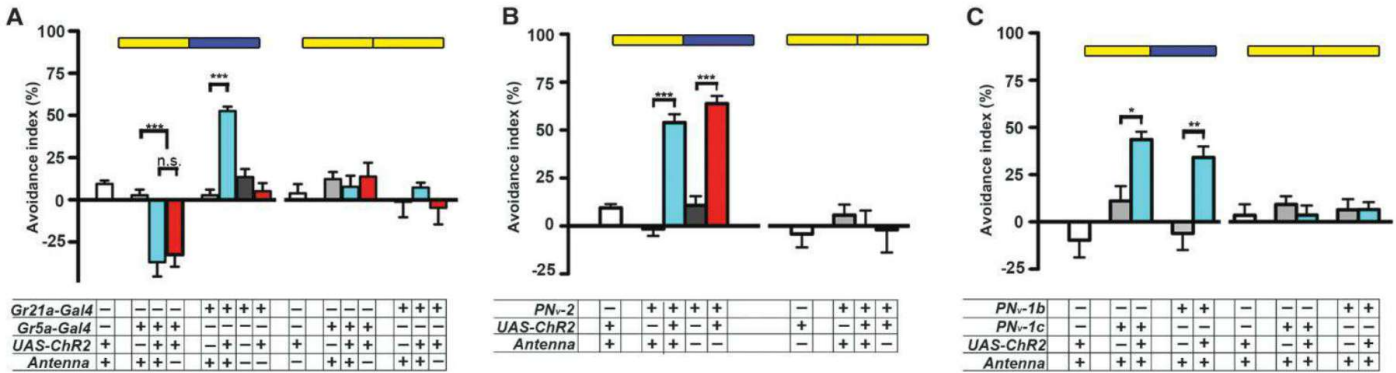
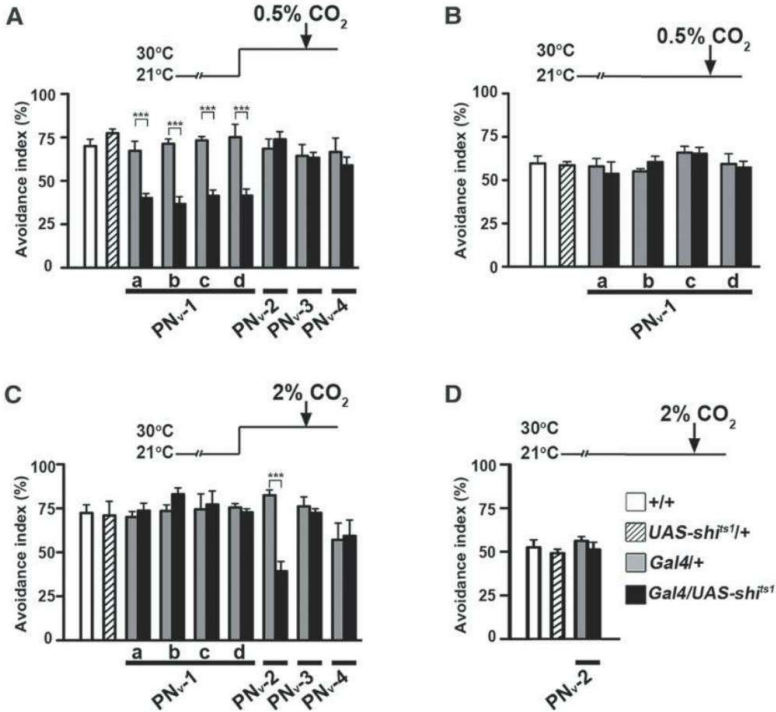


Fig. 3. Activation of PN_v-1 or PN_v-2 alone elicits avoidance behavior in an optogenetic T-maze. (A) Phototaxis responses to ChR2 activation of specific Gal4 neurons. (B) Avoidance responses to ChR2 activation of PN_v-2. E0044-Gal4>UAS-

ChR2 flies avoided the blue light. (C) Avoidance responses to ChR2 activation of PN_v-1. Blue and yellow bars indicate the blue and yellow LED arms, respectively. Each value is mean ± SEM (n = 6 to 8 experiments, *P < 0.05, ***P < 0.001).

and an air-cooling system (fig. S13C) (25). Intact antennae were necessary for avoidance behavior elicited by *Gr21a-Gal4* neurons but unnecessary for attraction behavior mediated by *Gr5a-Gal4* neurons (Fig. 3A). A similar avoidance response was observed upon optogenetic activation of PN_v-1 (using *VT31497-Gal4* and *VT1606-Gal4*) or PN_v-2 (using *E0044-Gal4*), thus mimicking the response to CO₂ exposure (Fig. 3, B and C). Avoidance behavior persisted without antennae. ChR2 activation of PN_v-3 or PN_v-4 did not elicit avoidance (fig. S14). Because neither blocking neurotransmission with *shi^{ts1}* nor silencing neural activity with *Kir2.1* completely abolished CO₂ avoidance, other PN_v types (fig. S2) may drive partial CO₂ avoidance behavior.

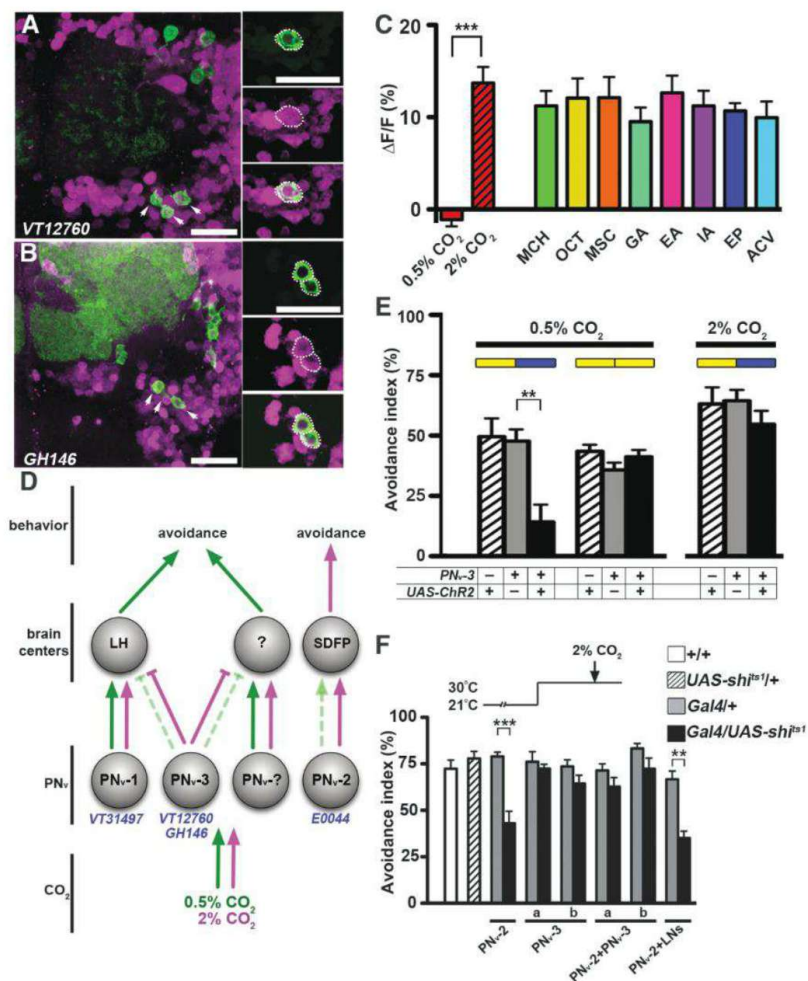
At 0.5% CO₂, PN_v-1 was functionally more responsive than was PN_v-2 (fig. S6B), explaining why only the former contributes to the low CO₂ avoidance response. However, 2% CO₂ activates

both PN_v classes. Why does 2% CO₂ avoidance require PN_v-2 but not PN_v-1? The PN_v-1 response remained higher at 2% CO₂ (fig. S6). Blocking neurotransmission from γ-aminobutyric acid (GABA)-releasing (GABAergic) local neurons (LNs) in the ALs did not impair 2% CO₂ avoidance (fig. S15). One possibility is that the PN_v-1 pathway is gated downstream at higher CO₂ levels. PN_v-3s are GABAergic (Fig. 4, A and B), but PN_v-1, PN_v-2, and PN_v-4 are not (fig. S16). PN_v-3 and PN_v-2 are highly activated by higher CO₂ concentrations (Fig. 1, Ee and Fe), and LH imaging revealed that PN_v-3 axon terminals were insensitive to 0.5% but highly sensitive to 2% CO₂ (Fig. 4C). This suggests a model in which PN_v-3 might inhibit and gate the PN_v-1 pathway in the LH. In this scenario, only the PN_v-1 pathway would be active at low CO₂ concentrations, and higher levels would recruit PN_v-3 and PN_v-2, blocking the PN_v-1 path-

way while activating the PN_v-2 pathway to elicit avoidance behavior (Fig. 4D).

This model makes two key predictions. First, optogenetic PN_v-3 activation should inhibit 0.5% but not 2.0% CO₂ avoidance. Second, PN_v-3 silencing should leave the PN_v-1 pathway active at 2% CO₂. Indeed, ChR2 activation of GABAergic PN_v-3 impaired avoidance to 0.5% but not 2% CO₂ (Fig. 4E), mimicking the effect of blocking PN_v-1 alone (Fig. 2A). Conversely, acutely blocking PN_v-3 neurotransmission did not impair avoidance responses, but PN_v-2 activity was no longer necessary for 2% CO₂ avoidance when GABAergic LNs in the AL were acutely blocked (Fig. 4F). Direct PN_v-3 inhibition to PN_v-1 is unlikely because PN_v-1 dendrites in the V-glomerulus (Fig. 1, Ae to De, and fig. S6) and axonal terminals in the LH (fig. S17) were not less responsive at high CO₂

Fig. 4. PN_v-3 gates CO₂ avoidance neural pathways. (A and B) PN_v-3 (arrows) labeled with *UAS-mCD8::GFP* (green) in *VT12760-Gal4* and *GH146-Gal4* were anti-GABA immunopositive (magenta). Scale bar, 25 μ m. **(C)** PN_v-3s exhibited broadly tuned responses to odorants. Each value is mean $\pm$ SEM ($n = 6$ samples, $***P < 0.001$). MSC, methyl salicylate; IA, isopentyl acetate; EP, ethyl propionate; EA, ethyl acetate; GA, geranyl acetate; ACV, apple cider vinegar. **(D)** Shunting inhibition model showing brain circuit routing for 0.5% (green) and 2% CO₂ (magenta). Excitatory and inhibitory stimuli are represented by arrows and "T" bars, respectively. Weak responses are represented as dashed lines. **(E)** Avoidance responses to 0.5 or 2% CO₂ when ChR2 in PN_v-3 (*VT12760-Gal4*) was activated by blue light. Blue and yellow bars indicate blue and yellow LED arms, respectively. Each value is mean $\pm$ SEM ($n = 6$ experiments, $**P < 0.01$). **(F)** Inhibition of PN_v-1 pathway by PN_v-3, not GABAergic LNs, during 2% CO₂ avoidance. Neurotransmission outputs of PN_v-2 in *E0044-Gal4* and PN_v-3 in *VT12760-Gal4* (a) and *GH146-Gal4* (b) were acutely blocked separately or together by *UAS-shi^{ts1}* at 30°C. Each value is mean $\pm$ SEM ($n = 6$ to 8 experiments, $***P < 0.001$).



levels when PN_v-3 was active. This model does not exclude the possibility of PN_v-3 inhibition on possible redundant PN_v pathways necessary for 0.5% CO₂ avoidance (Fig. 4D).

What benefit might be gained by channeling CO₂ signals through distinct PN pathways to elicit the same avoidance behavior? Although CO₂ is part of a deterrent signal issued by other flies under stress conditions (5), high levels of CO₂ can also indicate food sources. Some food odors can directly inhibit CO₂-sensitive neurons in the antenna (26). Behavior state is also critical, and in-flight *Drosophila* exhibits CO₂ attraction (27). The PN circuitry described here would allow concentration-dependent CO₂ avoidance. PN_v-3s may also modulate other olfactory behaviors because they innervate many glomeruli (Fig. 1F) and respond to multiple odors (Fig. 4C).

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25. The blue and yellow lights were balanced so that flies were naturally attracted to visible light distributed equally between two arms (fig. S13D). We validated effectiveness by expressing ChR2 in *Gr5a-Gal4* neurons (required for sucrose attraction) or *Gr21a-Gal4* neurons (required for CO₂ avoidance); as expected, these flies were attracted or repelled, respectively, by the blue light.
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Supplementary Materials

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Multisensory Control of Hippocampal Spatiotemporal Selectivity

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The hippocampal cognitive map is thought to be driven by distal visual cues and self-motion cues. However, other sensory cues also influence place cells. Hence, we measured rat hippocampal activity in virtual reality (VR), where only distal visual and nonvestibular self-motion cues provided spatial information, and in the real world (RW). In VR, place cells showed robust spatial selectivity; however, only 20% were track active, compared with 45% in the RW. This indicates that distal visual and nonvestibular self-motion cues are sufficient to provide selectivity, but vestibular and other sensory cues present in RW are necessary to fully activate the place-cell population. In addition, bidirectional cells preferentially encoded distance along the track in VR, while encoding absolute position in RW. Taken together, these results suggest the differential contributions of these sensory cues in shaping the hippocampal population code. Theta frequency was reduced, and its speed dependence was abolished in VR, but phase precession was unaffected, constraining mechanisms governing both hippocampal theta oscillations and temporal coding. These results reveal cooperative and competitive interactions between sensory cues for control over hippocampal spatiotemporal selectivity and theta rhythm.

Spatial navigation and hippocampal activity are influenced by three broad categories of stimuli: distal visual cues (1, 2); self-motion cues (3, 4), e.g., proprioception, optic flow, and vestibular cues (5); and other sensory cues (6, 7), e.g., olfaction (6, 8), audition (9), and somatosensation (10). Although the cognitive map is thought to be primarily driven by distal visual and self-motion cues (11), their contributions are difficult to assess in the real world (RW). Hence, we developed a noninvasive virtual reality (VR) (fig. S3) for rats, where the vestibular and other sensory cues did not provide any spatial information. Consequently, we will refer only to proprioception and optic flow as “self-motion cues,” and vestibular inputs will be treated separately. Place cells have been measured in VR in mice with their heads fixed (12, 13) and are thought to be similar in VR and RW, but this has not been tested. We used tetrodes to measure neural activity from the dorsal CA1 of six rats while they ran in VR or RW environments consisting of a linear track in the center of a square room with distinct distal visual cues on each of the four walls (Fig. 1A). The visual scene was passively turned when rats reached the end of the virtual track. The distal visual cues were nearly identical in VR and RW, but rats’ bodies were fixed in VR, which eliminated spatially informative other sensory cues and minimized both angular and linear vestibular inputs (fig. S4, see methods). Thus, the only spatially informative cues in VR during

track running were distal visual and self-motion cues, as defined above. During recordings, rats ran consistently along the track and reliably slowed before reaching the track end in both VR and RW (Fig. 1B, see methods). Although their running speed was somewhat lower in VR than RW, their behavioral performance was similar.

Clear, spatially focused and directionally tuned place fields, commonly found in RW (Fig. 1C), were also found in VR (Fig. 1D) (12). Almost all track-active putative pyramidal cells had significant spatial information in VR (96%) and RW (99%). Thus, distal visual and self-motion cues are sufficient to generate the hippocampal rate code or cognitive map. We then examined whether the cognitive maps were similar in VR and RW.

We measured the activities of 2119 and 528 putative pyramidal neurons in the baseline sessions, conducted in a sleep box, preceding the VR and RW tasks respectively (see methods). Of these, 45.5% were track-active in RW. In contrast, only 20.4% were track-active in VR (Fig. 1E and fig. S5). The VR track-active cells had only slightly smaller mean firing rates (VR: 2.71 ± 0.08 Hz, $n = 432$; RW: 3.06 ± 0.12 Hz, $n = 240$, $P < 0.05$), which is likely due to lower running speed (14). Although the track-active cells were measured during locomotion, we also investigated the place-cell activation during periods of immobility at the goal locations, where a similar reduction to one-half of the proportion of goal-active cells in RW was seen in VR, with no significant change in firing rates (table S1 and Fig. 1E).

The firing rate maps of place cells were slightly less stable in VR (stability index 0.80 ± 0.01 , $n = 432$) than RW (0.87 ± 0.01 , $n = 240$). Hence, all subsequent comparisons were made across only the 392 and 227 track-active, stable cells (fig. S6, see methods) in VR and RW respectively. Place fields were 26% wider ($P < 10^{-10}$) in VR (55.8 ± 1.2 cm, $n = 482$) compared to RW (44.3 ± 1.4 cm, $n = 365$). As a result, spatial selectivity was 22% lower in VR (Fig. 1F, fig. S7, and table

S1). Further, 90% (75%) of track-active cells had at least one place field in RW (VR), and place fields had comparable and significantly asymmetric shapes in both VR and RW such that the firing rates were higher at the end of a place field than at the beginning (table S1) (12, 15, 16).

We then investigated the directionality of place cells in VR. As in RW (14), the majority of place cells were directional, spiking mostly in one running direction (Fig. 1, C and D). In fact, the distributions of directionality index were identical (fig. S7 and table S1). But, bidirectional cells (40% in VR and 43% in RW), which had substantial activity along both running directions, showed different behavior in RW and VR (Fig. 2A). Bidirectional cells fired around the same absolute position on the track in both running directions in RW (Fig. 2A) (6, 17), expressing a position code. In contrast, bidirectional cells in VR fired around the same distance from the start position in both running directions (Fig. 2A), indicative of a disto-code. The position code index (see methods) was significantly positive in RW but significantly negative in VR, indicating a position code in RW and its absence in VR (Fig. 2B). Exactly the opposite was true for the disto-code (Fig. 2C). Analysis of trials run at different speeds showed that spiking was more correlated with distance along the track than the duration of running, suggesting that it is distance run rather than time that determined these cells’ activity (fig. S8).

To assess these results at the population level, we performed population vector overlap analysis (Fig. 2, D and E, and fig. S9; see methods). The population of bidirectional cells spiked around the same absolute position on the track in two movement directions in RW, indicated by a significant increase in population vector overlap along the -45° diagonal (Fig. 2D). There was no significant overlap along $+45^\circ$ (at the same distance along two directions) in RW. The opposite was true in VR (Fig. 2E), with significant overlap along $+45^\circ$ and no significant overlap along -45° . Thus the disto- and position codes were present at the population level in VR and RW respectively, but not vice versa.

Examination of the same cells recorded in both VR and RW revealed that their mean firing rates, spatial information, and directionality index were correlated between the environments, but the position of their place fields was unrelated (figs. S10 and S11). In the same bidirectional cells, the disto-code index was greater in VR than in RW, and the position code index was greater in RW than VR (fig. S12).

Having examined the rate-code, we investigated the temporal features (12, 16, 18–20) of place cells. The frequency of theta rhythm (see methods) in the local field potential (LFP) during locomotion was reduced in VR compared to RW (Fig. 3A). Despite this, VR cells showed clear phase precession, comparable to RW (Fig. 3, B and C). Indeed, across the ensemble of data, the LFP theta frequency within place fields was

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8.7% lower in VR compared to RW (Fig. 3D), yet there was no significant difference in the quality of phase precession between the two conditions (Fig. 3E). As a result, the frequency of theta modulation of spiking of place cells (12, 16, 18–21) was also significantly reduced in VR (fig. S13). Thus the frequency of theta rhythm is different in VR and RW, but it has no impact on the hippocampal temporal code.

Theta frequency was significantly lower in VR than RW at all running speeds (Fig. 4, A and B). Across the ensemble, theta frequency

increased with running speed in RW but this was abolished in VR (Fig. 4B). We thus computed the speed dependence of theta frequency for each electrode within each session (see methods). Noisy but clear speed-dependent increase in theta frequency was found within single LFP data in RW (Fig. 4C), but not in VR (Fig. 4D). These calculations of the single cycle theta frequency could be influenced by noise, which could especially distort the low-amplitude theta and provide erroneous results. Hence, we restricted the analysis to only high-amplitude theta cycles

(see methods). A large majority (85.4%) of LFPs in RW showed significant correlation between running speed and theta frequency but this was abolished in VR (Fig. 4E and figs. S14 and S15). In contrast, theta amplitude showed identical correlation with running speed in both conditions (Fig. 4F).

The key findings were qualitatively true within each animal. In particular, within each animal the disto (position) index was positive (negative) in VR, and negative (positive) in RW, a substantially greater fraction of cells were active in RW than VR and theta frequency was greater in RW than VR. Further, control experiments show that the disto-code was not driven by salient reward, indicating cues in the environment (figs. S16 and S17); was present in both passive and active turning protocols (fig. S18); and was consistent between slow and fast trials in VR (fig. S19).

These results reveal several important aspects of the sensory mechanisms governing hippocampal rate and temporal codes. Comparable levels of spatial selectivity and firing rates of track-active cells in VR and RW, as well as comparable strength of temporal code, all show that a robust cognitive map can indeed be formed with only distal visual and self-motion cues providing spatial information. However, these cues alone do not determine the location of place fields between VR and RW, as shown by the differences in ratemaps of the same cells.

The VR and RW environments had similar dimensions and nearly identical distal visual cues, and the rats' behavior was similar in the two conditions. Thus, these factors are unlikely to cause the observed differences in place cell activity between VR and RW. Several other factors could potentially contribute, in particular, the absence of spatial information in vestibular and other sensory cues, or conflicts of other sensory cues with distal visual or self-motion cues in VR. One potential consequence of conflicting cues could be reference frame switching (between virtual and real space) occurring randomly along the track, resulting in loss of spatial selectivity in VR. The overwhelming presence of significant spatial information in cells recorded in VR argues against this possibility. Instead, these results suggest that additional sensory cues present in RW are necessary for the activation of a subpopulation of CA1 place cells, such that their removal reduces the activation of place cells without altering the firing rates of active cells. In VR, spatial information is provided by only distal visual and self-motion cues, which likely change more slowly than spatially informative proximal cues, such as odors along the track in RW. This could make place fields wider in VR and therefore reduce their information content.

The switch in coding observed in the bidirectional cell population is analogous to a previous report of different hippocampal cell response types (4), which implies either distinct classes of principal cells or alternate responses from the

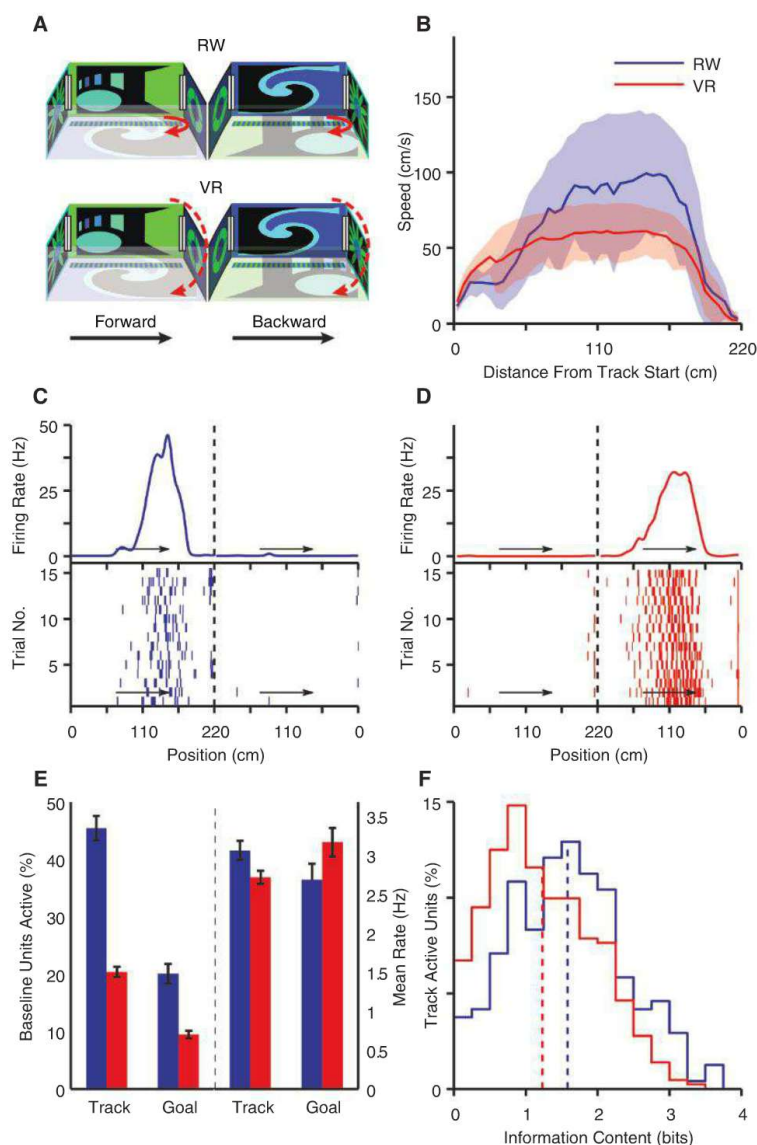


Fig. 1. Large reduction of track-active cells in VR without comparable reduction in firing rates. (A) Schematic of the task environment and distal visual cues in VR and RW. Rats turned themselves around in RW, whereas the scene was passively reversed in VR. (B) Running speed (means $\pm$ SD) of rats as a function of position on a 2.2-m-long linear track for RW (blue) and VR (red). Similar color scheme is used throughout. Although the rats were faster in RW, their behavior was similar, reliably reducing speed before reaching the end of the track ($n = 49$ sessions in RW, $n = 128$ sessions in VR). (C) Example of a directional, stable place cell recorded in RW with firing rate (top), and raster plot (bottom). Arrows indicate running direction. (D) A similar place cell recorded in VR. (E) Comparison of activation ratio and firing rates of active cells on track (RW: 45.5%, 3.06 ± 0.12 Hz; VR: 20.4%, 2.71 ± 0.08 Hz) and at goal (RW: 20.1%, 2.68 ± 0.20 Hz; VR: 9.5%, 3.16 ± 0.18 Hz). (F) Spatial information content across 432 track-active cells in VR (1.23 ± 0.03 bits, $n = 432$) was significantly lower (22%, $P < 10^{-7}$) than in 240 RW cells (1.58 ± 0.05 bits, $n = 240$).

same cells depending on the task. The latter seems to be more likely in our data because comparisons between VR and RW show that the same hippocampal cell can acquire a different form of representation given a different subset of inputs. We cannot rule out the possibility that higher-order cognitive processes may influence the place cell code; however, it is likely that sensory inputs play a key role in our results. Similar levels of directional tuning in VR and RW suggest that distal visual cues, which are the only cues that differ along two movement directions on the track in VR, are sufficient to generate directional firing on a linear track. Vestibular inputs are not required for generating spatially selective, directional place cells, in contrast to previous lesion-based studies (5). The vestibular cues are present and identical along two movement directions in RW; therefore, their presence, not their absence, should contribute to a disto-code (figs. S20 and S21). Bidirectional cells in RW exhibit position code (17), which is enhanced by the addition of odors and textures (6). These points suggest proximal cues in RW are the most likely to generate the position code. Bidirectional cells in VR exhibit disto-code and are likely governed by self-motion cues, which are the only cues that

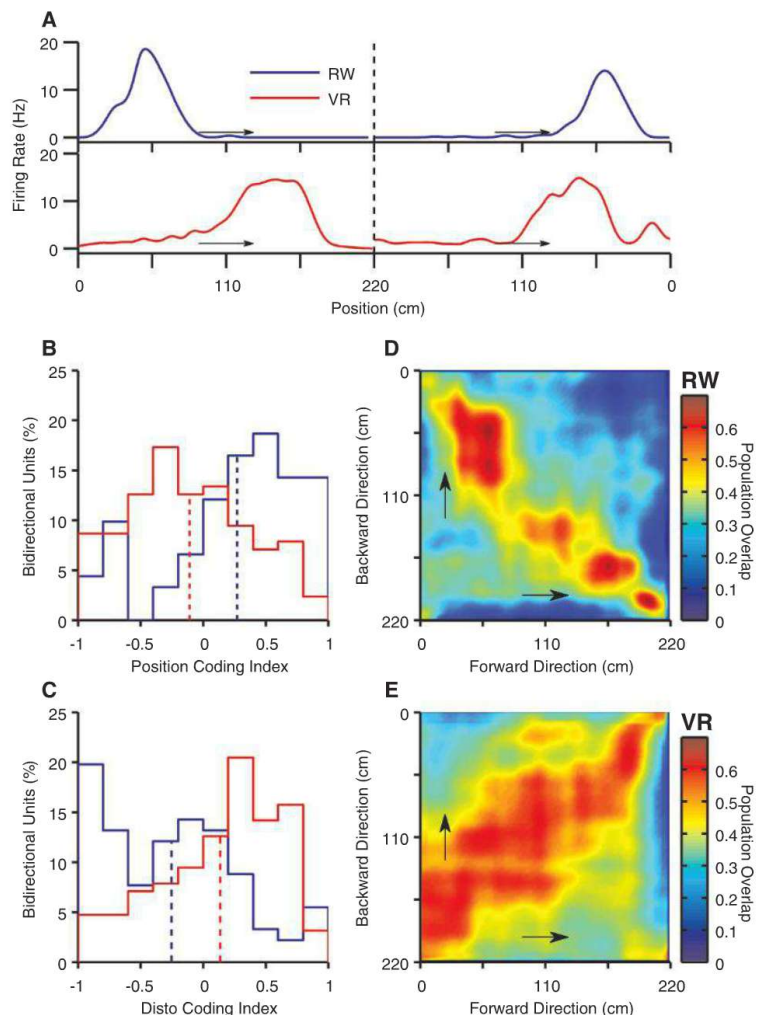
are similar along the two movement directions. Finally, we hypothesize that other sensory cues present in RW have a veto power over self-motion cues in determining the bidirectional code.

Although disto-code has been reported on a single-unit level in RW (3, 22), we do not see significant disto-code in the RW population. Further, the effect of self-motion cues on the hippocampal population code and the suggested competitive interaction between self-motion and other sensory cues are surprising. Such competitive effects between different sensory modalities may be driven by inhibitory mechanisms across multimodal inputs, as seen recently in the primary visual cortex (23), which suggests their broader applicability. We hypothesize that some other sensory cues reach CA1 via the lateral entorhinal cortex (LEC), because LEC neurons respond to local cues such as objects (24), whereas distal visual and self-motion cues reach CA1 via the medial entorhinal cortex (MEC) grid cells, which are influenced by these cues (25). Consistently, both LEC and MEC project directly and indirectly to CA1 and differentially modulate CA1 activity in vivo during sleep (26) and engage local inhibition (27). The competitive interactions governing the behavior of bidirectional cells in VR and RW

may therefore be the result of inhibitory interactions between the LEC and MEC pathways.

Theta frequency was significantly reduced in VR, corroborating earlier results that vestibular inputs contribute to theta frequency (28), and its speed dependence was abolished. On the other hand, theta power had similar speed dependence in VR and RW, which suggested that theta power is largely governed by distal visual and self-motion cues. Despite the large changes in theta frequency and its speed dependence, phase precession was intact in VR (12), and its quality was identical in RW and VR, which indicated that distal visual and self-motion cues are sufficient to generate a robust temporal code. Our results place restrictions on theories of phase precession that depend on the precise value of theta frequency or its speed dependence (18, 29–31). Instead, they favor alternative mechanisms that are insensitive to these phenomena (15, 16, 19) and that apply equally to networks with diverse connectivity patterns, such as the entorhinal cortex and CA1, and, hence, do not require recurrent excitatory connections (15, 16). These results thus provide insight about how distinct sensory cues cooperate and compete to influence theta rhythm and hippocampal spatiotemporal selectivity.

Fig. 2. Bidirectional place cells exhibit position code in RW but disto-code in VR. (A) Firing rate maps along both running directions for bidirectional cells in RW (top) and VR (bottom). (Top) A position-coding cell firing at the same position in both running directions. (Bottom) A disto-coding cell firing at the same distance in both directions. **(B)** Position code index is significantly positive in RW (0.27 ± 0.05 , $P < 10^{-6}$, $n = 91$) but significantly negative in VR (-0.11 ± 0.04 , $P < 0.05$, $n = 127$). **(C)** The disto-code index is significantly positive in VR (0.14 ± 0.04 , $P < 0.001$, $n = 127$) but significantly negative in RW (-0.25 ± 0.06 , $P < 10^{-4}$, $n = 91$). The position code index is significantly greater in RW than VR ($P < 10^{-7}$) whereas disto-code index is significantly greater in VR ($P < 10^{-6}$). **(D)** Similarity of the population of 91 bidirectional cells in RW between two movement directions was computed using the population vector overlap (see methods). Each colored pixel shows the vector overlap between two positions in opposite running directions. Note the clear increase in overlap along the -45° diagonal indicating spiking at the same position. **(E)** As in (D), for the population of 127 bidirectional cells in VR. Note the clear increase in overlap along the $+45^\circ$ diagonal, indicating spiking at the same distance in both running directions.



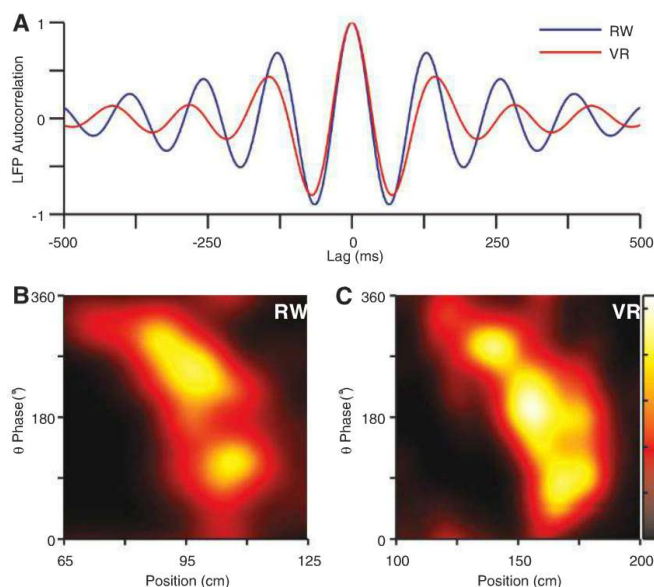


Fig. 3. Reduced theta frequency in VR without significant change in phase precession. (A) Autocorrelation function of sample hippocampal LFPs in RW and VR showing significant increase in theta period in VR. Both LFPs are recorded from same electrode on the same day. (B) Representative place field in RW showing clear phase precession. (C) Representative place field in VR showing clear phase precession. (D) In-field theta frequency of place fields in VR (7.53 ± 0.02 Hz, $n = 251$) was significantly less (8.7%, $P < 10^{-10}$, $n = 204$) than that of place fields in RW (8.25 ± 0.03 Hz, $n = 204$). (E) Quality of phase precession, measured by position-phase linear-circular correlation, in RW (0.33 ± 0.01) was not significantly different ($P = 0.8$) from precession in VR (0.33 ± 0.01).

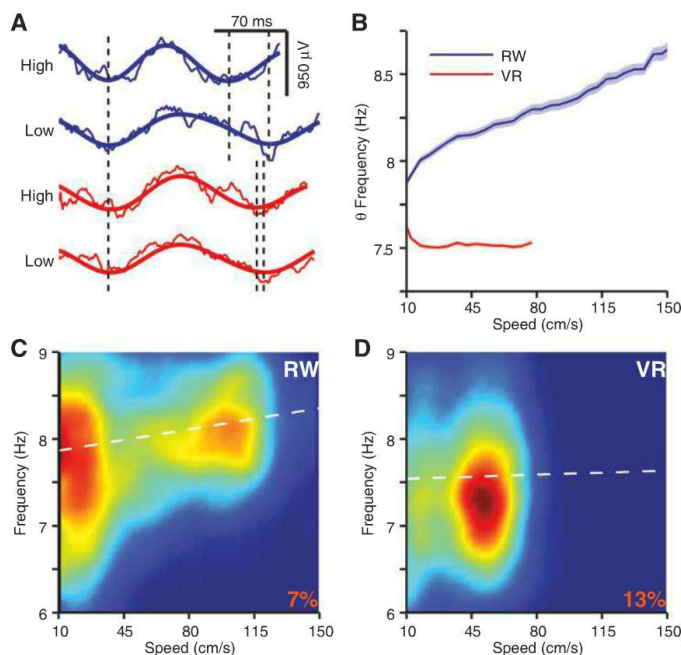


Fig. 4. Absence of theta-frequency speed dependence in VR. (A) Sample theta cycles during high (>50 cm/s) and low (<10 cm/s) running speed in RW and VR from the same electrode seen in Fig. 3A. Bold lines are filtered between 4 and 12 Hz, narrow lines are unfiltered traces (see Fig. S15). (B) Population average (mean $\pm$ SEM) speed dependence of theta frequency from 287 LFPs in RW and 681 LFPs in VR. (C) Density map of individual theta-cycle frequencies and corresponding speeds from a single LFP in RW (correlation = 0.13, $P < 10^{-10}$). (D) As in C, for the same electrode in VR on the same day (correlation = 0.01, $P = 0.48$). (E) The population of LFPs in RW shows significant correlation between theta frequency and speed (0.21 ± 0.01 , $P < 10^{-10}$), whereas the population of LFPs in VR shows no significant correlation (-0.01 ± 0.01 , $P < 0.01$). (F) Theta cycle amplitude is similarly ($P = 0.8$) correlated with speed in both RW (0.16 ± 0.01) and VR (0.16 ± 0.01).

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designed the experiment; and M.R.M. participated in all of the above.

Supplementary Materials

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Subangstrom Resolution X-Ray Structure Details Aquaporin-Water Interactions

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Aquaporins are membrane channels that facilitate the flow of water across biological membranes. Two conserved regions are central for selective function: the dual asparagine-proline-alanine (NPA) aquaporin signature motif and the aromatic and arginine selectivity filter (SF). Here, we present the crystal structure of a yeast aquaporin at 0.88 angstrom resolution. We visualize the H-bond donor interactions of the NPA motif's asparagine residues to passing water molecules; observe a polarized water-water H-bond configuration within the channel; assign the tautomeric states of the SF histidine and arginine residues; and observe four SF water positions too closely spaced to be simultaneously occupied. Strongly correlated movements break the connectivity of SF waters to other water molecules within the channel and prevent proton transport via a Grotthuss mechanism.

Aquaporins are water transport facilitators found in all kingdoms of life (1). They are primarily responsible for water homeostasis within living cells, although a subset of aquaporins also facilitates the flow of other small polar molecules, such as glycerol or urea. As with any membrane transport facilitator, aquaporins have evolved to be highly selective for their transported substrate without binding water so strongly that transport is inhibited. In addition to excluding hydroxide (OH⁻) and hydronium (H₃O⁺) ions, aquaporins must also prevent proton transport via a Grotthuss mechanism (2, 3) in which protons are rapidly exchanged between hydrogen bonded water molecules.

Crystal structures of bacterial (4, 5), archaeal (6), yeast (7), plasmodium (8), plant (9), mammalian (10–12), and human (13–15) aquaporins have established that these channels contain six transmembrane α helices and associate as homotetramers. A seventh pseudo-transmembrane helix

is formed by loops B and E, which fold as aligned half-helices that insert from opposite sides of the membrane and place the conserved dual asparagine-proline-alanine (NPA) signature motif near the center of the water pore (Fig. 1). Transport specificity is defined by the aromatic and arginine selectivity filter (SF) (16, 17), which is located near the extracellular pore entrance and forms the narrowest portion of the channel.

Several models accounting for the ability of aquaporins to impede the passage of protons have emerged from structural arguments (13), molecular dynamics (MD) investigation of water structure and dynamics (17, 18), and computational studies characterizing the energetics associated with explicit transfer of protons across the channel (19, 20). These studies assert different microscopic mechanisms for excluding protons, including electrostatic repulsion (19, 21–23), configurational barriers (17), and desolvation penalties (24), and they consistently report the NPA region, where the macrodipoles of the two half-helices formed by loops B and E focus a positive electrostatic potential, as the main barrier against proton transport (17–24). This creates an electrostatic barrier to proton transport (21) and orients the water molecule's dipole moment near the NPA motif, such that the order of oxygen and hydrogen atoms do not support proton exchange via a Grotthuss mechanism (17, 18). Although intuitively appealing, this picture does not explain why muta-

tions within the NPA motifs that diminish this positive electrostatic barrier facilitate the transport of sodium ions, but not protons (25, 26); nor is it evident why mutations within the SF can allow the channel to conduct protons (27, 28).

To further examine the underlying mechanism of facilitated, selective water transport, we optimized crystals of Aqp1 (7), the sole aquaporin of *Pichia pastoris*, and determined its crystal structure to 0.88 Å resolution (29), recovering *R*-factor and *R*_{free} values of 10.3% and 10.7%, respectively (table S1). The electron density associated with the dual-NPA-aquaporin signature motif is illustrated in Fig. 2. At subangstrom resolution, the conformations of the two NPA asparagine residues (Asn¹¹² and Asn²²⁴) are uniquely assigned because the $2mF_{\text{obs}} - DF_{\text{calc}}$ electron density is delocalized across the carbon-oxygen double bond of these side chains (Fig. 2, A and B, blue mesh), whereas that for the side-chain nitrogen atom is more localized: *m* is the figure of merit, and *D* is estimated from coordinate errors. Electron density associated with Glu⁵¹ (fully delocalized) and Gln¹³⁷ (partially delocalized) highlights how delocalized density can be distinguished at this resolution (fig. S1). It is noteworthy that residual $mF_{\text{obs}} - DF_{\text{calc}}$ difference electron density, from a hydrogen omit map (Fig. 2, A and B, green mesh), reveals electron clouds associated with all four proton-donor interactions of the Nδ atoms of the dual-NPA asparagine residues. H-bond donor interactions of Asn²²⁴:Nδ to water molecule 6 (Wat6) (peak maximum 0.42 e/Å³) (Fig. 2A) and to the carbonyl oxygen of Leu¹¹¹, as well as H-bond donor interactions of Asn¹¹²:Nδ to Wat7 (peak maximum 0.48 e/Å³) (Fig. 2B) and to the carbonyl oxygen of Leu²²³, are all resolved. These observations confirm that H-bond donor interactions from the NPA motifs constrain the orientation of passing water molecules (13, 17, 18). No modeled water has H-bond interactions with both NPA asparagines, as is often depicted (13, 17, 19, 22, 23), and a water molecule at this position cannot be the critical ingredient preventing Grotthuss proton transport.

MD simulations have predicted that water molecules adopt a bipolar orientation in the two halves of the channel, such that proton-donor interactions systematically point away from the NPA region, which disfavors Grotthuss proton exchange (13, 17, 18). In the cytoplasmic half-channel, residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density

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reveals that Wat8 donates an H bond to Wat9 (peak maximum $0.73 \text{ e}/\text{\AA}^3$) (Fig. 2C); Wat9 to Wat10 (peak maximum $0.70 \text{ e}/\text{\AA}^3$); Wat10 to the hydroxyl group of Tyr³¹ (peak maximum $0.79 \text{ e}/\text{\AA}^3$), and weaker residual density suggests an H bond

from Wat7 to Wat8 (peak maximum $0.30 \text{ e}/\text{\AA}^3$). A positive peak located almost exactly between Wat6 and Wat7 (peak maximum $0.50 \text{ e}/\text{\AA}^3$) suggests that these two waters donate an H bond to each other with approximately equal probability,

which illustrates that H-bond directionality is not imposed at the very center of the channel where the positive electrostatic potential is at its maximum. The polarity of water-water H bonds in the extracellular half-channel is difficult to assign because of larger anisotropic motions of Wat5 and Wat6 (B-factors of 15.0 and $13.9 \text{ \AA}^2$, respectively) relative to water molecules in the cytoplasmic half-channel (B-factors of 9.7 to $12.1 \text{ \AA}^2$). Nevertheless, all assigned interactions are consistent with the proposed bipolar distribution of water-water H bonds (13, 17, 18), and this polarization does not depend on the presence of a water molecule that simultaneously accepts H bonds from both NPA asparagines.

Within the SF, His²¹² and Arg²²⁷ are conserved among water-selective aquaporins. The side-chain conformation of His²¹² is apparent from the stronger electron density visible for its two nitrogen atoms (Fig. 3). Residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density from a hydrogen omit map reveals that N δ of His²¹² is protonated and donates an H bond to the carbonyl oxygen of Leu²⁰⁸ (visible at $0.42 \text{ e}/\text{\AA}^3$), whereas Ne is not protonated (Fig. 3A), which indicates its tautomeric state (fig. S2A). Similarly, the tautomeric state of Arg²²⁷ (fig. S2B) can be assigned because the covalent bond from C ζ of Arg²²⁷ to N η 2 (the nitrogen atom closest to the water channel) is most conjugated (Fig. 3A). Consistent with this assignment, electron clouds associated with the three protons of Ne and N η 1

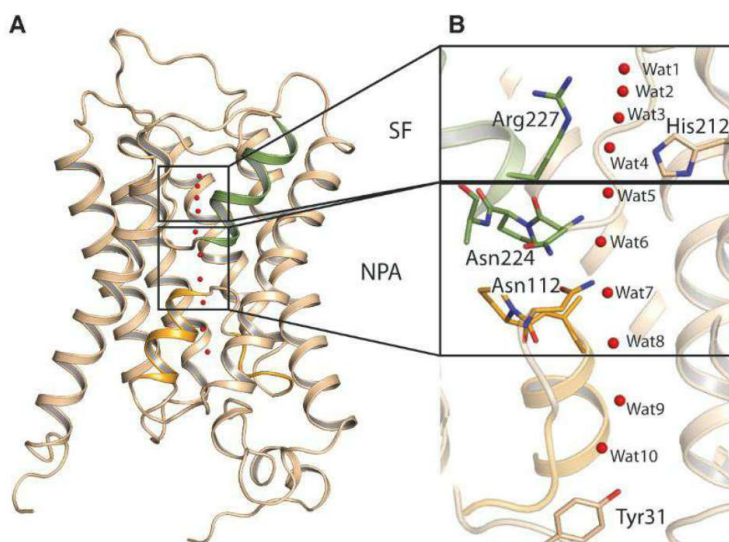
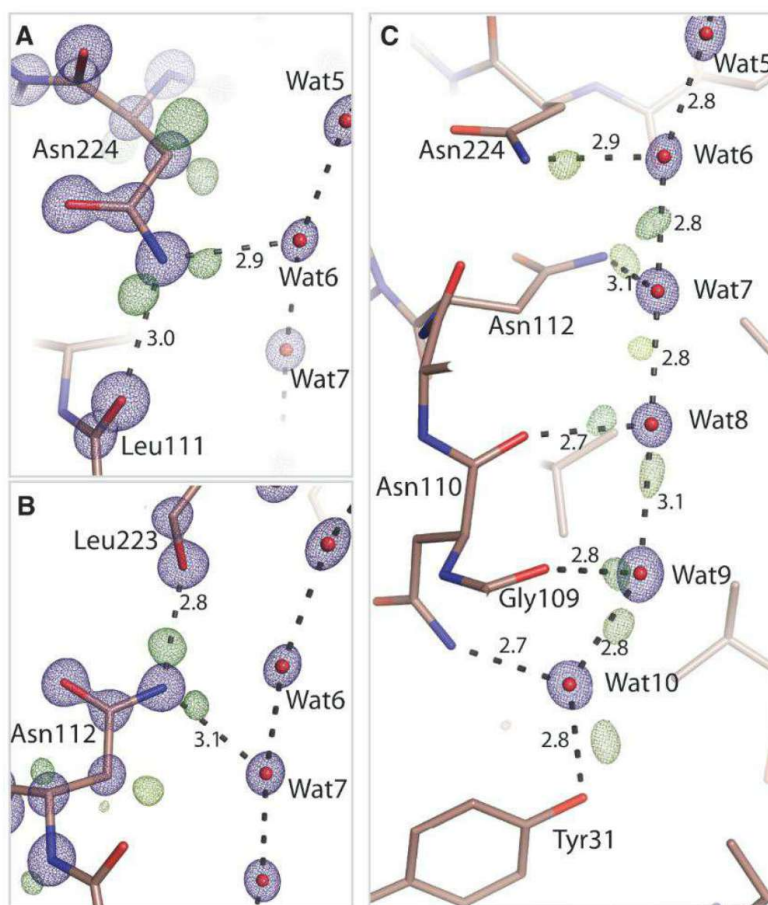


Fig. 1. Fold of Aqp1. (A) The six transmembrane helices and the seventh pseudo-transmembrane helix formed by loops B (orange) and E (green). (B) Water molecule positions within the channel (red spheres). The dual-NPA-aquaporin signature motif (bottom box) and the SF (top box) are highlighted.

Fig. 2. Electron density within the NPA and cytoplasmic regions of the Aqp1. (A) The $2mF_{\text{obs}} - DF_{\text{calc}}$ (blue, contoured at $4.3 \text{ e}/\text{\AA}^3$) and $mF_{\text{obs}} - DF_{\text{calc}}$ (green, contoured at $0.33 \text{ e}/\text{\AA}^3$) electron density associated with Asn²²⁴. (B) The $2mF_{\text{obs}} - DF_{\text{calc}}$ (blue, contoured at $4.3 \text{ e}/\text{\AA}^3$) and $mF_{\text{obs}} - DF_{\text{calc}}$ (green, contoured at $0.33 \text{ e}/\text{\AA}^3$, yellow-green at $0.26 \text{ e}/\text{\AA}^3$) electron density associated with Asn¹¹². Delocalized $2mF_{\text{obs}} - DF_{\text{calc}}$ density connects the dual-NPA asparagine C γ and O δ atoms, whereas that associated with N δ is more localized. Residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density indicates H-bond donor interactions with passing water molecules. (C) The $2mF_{\text{obs}} - DF_{\text{calc}}$ electron density (blue, contoured at $4.3 \text{ e}/\text{\AA}^3$) illustrates the position of water molecules, and $mF_{\text{obs}} - DF_{\text{calc}}$ residual electron density (brown-green contoured at $0.59 \text{ e}/\text{\AA}^3$, dark green at $0.39 \text{ e}/\text{\AA}^3$, yellow-green 0.26 to $0.33 \text{ e}/\text{\AA}^3$, and light green contoured at $0.15 \text{ e}/\text{\AA}^3$) indicates water H-bond interactions within the aquaporin channel.



are visible (0.48, 0.42, and 0.36 peak maxima $e/\text{\AA}^3$, respectively) (Fig. 3A), but no residual density is visible for the protons of N η 2, presumably because the associated electrons are more tightly drawn to a net-positive charge on N η 2. This concentration of positive charge on the nitrogen atom closest to the pore maximizes the electrostatic repulsive effect of Arg²²⁷ on hydronium ions and thereby helps inhibit their passage.

When two water molecules were built into the electron density between His²¹² and Arg²²⁷ (fig. S3), they had significantly lower $2mF_{\text{obs}} - DF_{\text{calc}}$ maxima (5.0 and 5.2 $e/\text{\AA}^3$) than all other water molecules within the aquaporin pore (~ 7.0 to $8.0 e/\text{\AA}^3$), and strong residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density peaks (maxima of 1.4 and 1.9 $e/\text{\AA}^3$) arose adjacent to these water molecules (Fig. 3B). Placement of two additional water molecules and unrestrained refinement of their crystallographic occupancies and B-factors revealed four stable water positions within the SF with complementary occupancies of 66% (Wat2 and Wat4, $2mF_{\text{obs}} - DF_{\text{calc}}$ peak maxima of 5.2 and 5.0 $e/\text{\AA}^3$, respectively) (Fig. 3) and 34% (Wat1 and Wat3, $2mF_{\text{obs}} - DF_{\text{calc}}$ peak maxima of 2.4 and 3.2 $e/\text{\AA}^3$, respectively). Given their small separation ($\sim 1.5 \text{\AA}$) (Fig. 3A), it is not possible for all four water positions to be occupied simultaneously. Although MD simulations predict only one high-probability water position within the SF (between Wat2 and Wat3) when averaged over an entire trajectory (Fig. 4B), specific snapshots capture these crystallographic water configurations (Fig. 4, D and E) and illustrate how water molecules move pair-wise through the SF while maintaining H-bond interactions with His²¹² and Arg²²⁷. This picture is analogous to the structural mechanism of ion transport through potassium channels, for which partial crystallographic occupancy of four K⁺-binding sites within its selectivity filter led to the proposal that potassium ions progress pair-wise through a sequence of four binding sites (30, 31). As argued for potassium channels (31), the similar crystallographic occupancy of the four aquaporin SF water positions implies very little energy difference between binding configurations 1,3 and 2,4, which is optimal for maximizing the water conduction rate.

Both high- and low-occupancy SF water positions have H-bond interactions with the same atoms: with N η 2 of Arg²²⁷ and the carbonyl oxygen of Gly²²⁰ for Wat1 and Wat2; and N δ of Arg²²⁷, Ne of His²¹², and the carbonyl oxygen of Ala²²¹ for Wat3 and Wat4 (Fig. 3A). MD snapshots illustrate how this geometry achieves exceptional water selectivity, because all four H-bond donor and acceptor interactions are filled as water moves through the SF (Fig. 4C). The presence of four closely spaced water-selective sites optimizes the aquaporin SF's ability to discriminate water from other small molecules. Hydroxide ions, in particular, suffer a geometric penalty, because they cannot simultaneously donate H bonds to the backbone hydroxyl of Ala²²¹ and to Ne of

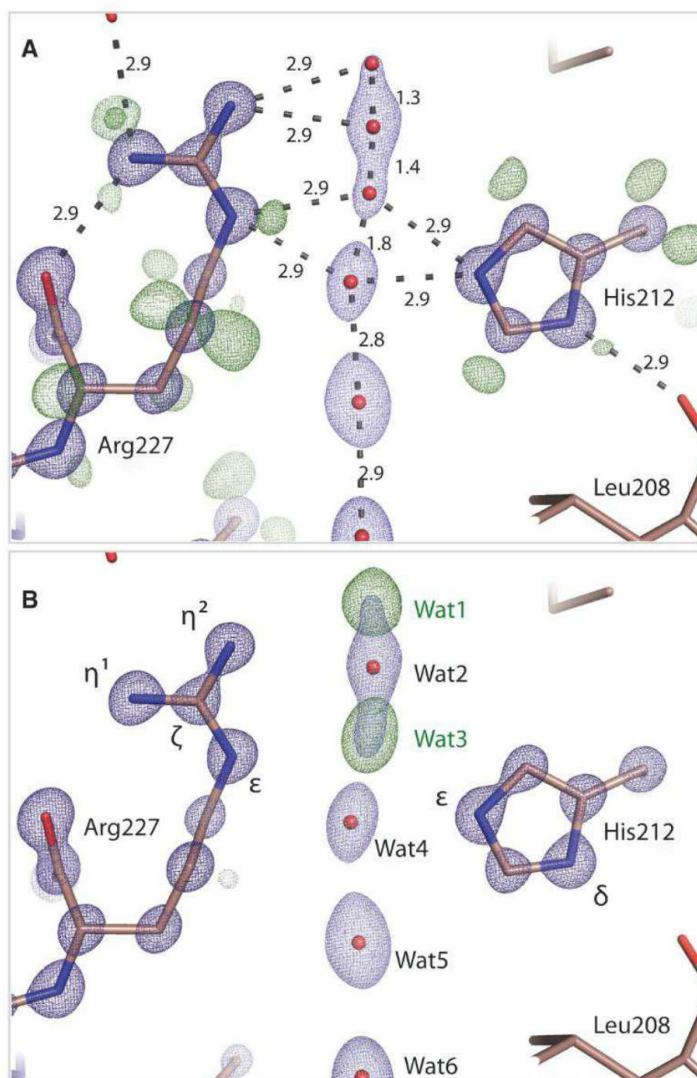


Fig. 3. Electron density within the Aqp1 SF. (A) The $2mF_{\text{obs}} - DF_{\text{calc}}$ (dark blue contoured at $4.3 e/\text{\AA}^3$ and light blue at $1.9 e/\text{\AA}^3$) and residual $mF_{\text{obs}} - DF_{\text{calc}}$ (dark green contoured at $0.42 e/\text{\AA}^3$ and light green contoured at $0.33 e/\text{\AA}^3$) electron density associated with His²¹², Arg²²⁷, and water molecules within the SF. Atomic separations (Å) are indicated. Residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density reveals that N δ of His²¹² is protonated, whereas N ϵ is not. Connected $2mF_{\text{obs}} - DF_{\text{calc}}$ electron density suggests that the Arg²²⁷ covalent bond from C ζ to N η 2 is preferentially conjugated. Four closely spaced water molecules are modeled within the SF with complementary occupancy (66% occupancy, positions 2 and 4; 34% occupancy, positions 1 and 3). **(B)** The $mF_{\text{obs}} - DF_{\text{calc}}$ omit electron density map calculated when waters 1 and 3 are removed from the structural model (dark green contoured at $0.65 e/\text{\AA}^3$). Positive electron-density features associated with these waters are the strongest within the channel.

His²¹². Conversely, all H-bond interactions are distorted from ideal water geometry (fig. S4), and this avoids binding water too tightly, such that efficient transport is compromised.

MD simulations reproduce the crystallographic positions of waters 5 and 7 to 10 as the most probable channel water positions at any given moment (Fig. 4A), whereas the location of Wat6 is more diffuse, consistent with its higher anisotropic crystallographic B-factor. The H-bond connectivity of water molecules is significantly perturbed at both the NPA and the SF regions of the channel, preventing Grotthuss proton transport (fig. S5). Within the NPA region, this disruption

is accompanied by a reduction in the correlated motion of water molecules (Fig. 4B), because one water molecule (corresponding to Wat6) rapidly alternates its H-bond interactions between neighboring waters on either half of the channel. This finding is consistent with the crystal structure, because residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density between Wat6 and Wat7 is significantly weaker than that observed between waters 8, 9 and 10, and no residual density is visible between Wat5 and Wat6 to suggest a well-defined H bond (Fig. 2C). In contrast, water molecules move pair-wise through the SF in a highly correlated manner (Fig. 4B), and their connectivity to water molecules

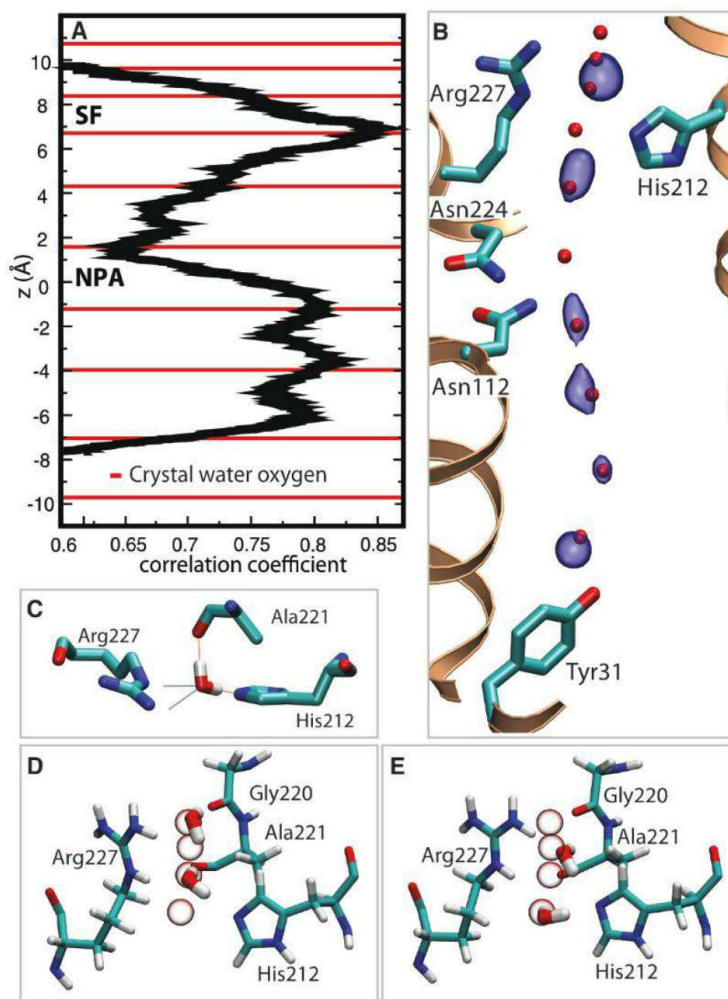


Fig. 4. Molecular dynamics simulations of water movements in Aqy1. (A) Plot illustrating the correlation of movements of adjacent water molecules. Strongly correlated movements arise in the SF and in the cytoplasmic half of the channel. The positions of oxygen atoms of crystallographic waters are indicated as red lines. (B) Surface representation (blue) of the most probable positions of water molecules (averaged over the final 15 ns of a 20-ns trajectory) superimposed on the crystallographic water positions (red spheres). (C) Snapshot of a water molecule within the SF with all four H-bond interactions occupied. (D and E) Snapshots corresponding to 1,3 and 2,4 water occupancy of the SF, which indicate the pair-wise movement of water molecules in this region. All four closely spaced SF crystallographic water positions are indicated as white spheres.

outside of the SF is weak. Correlated motions within the cytoplasmic half of the channel emerge from well-defined water-water H-bond interactions (Fig. 2C), whereas the correlations observed within the SF appear to be dictated by protein-water H-bond interactions. In addition to electrostatic effects, a disruption of the highly constrained SF water structure (Fig. 3) can explain why the mechanism of proton exclusion is sensitive to mutation of the conserved SF arginine and histidine residues (27, 28). These findings illustrate how evolution has fine-tuned the water channel geometry so as

to optimize protein function, suppressing proton transport without compromising water flux.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6138/1346/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 and S2
References (32–49)

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Biology Watches The Cloud

As genome sequencers churn out terabytes of new data daily, researchers are increasingly turning to an information-handling strategy already favored by internet companies: cloud computing. **By Alan Dove**



"If your data involves more than a hundred terabytes that needs to be shared amongst collaborators, I think you'll get the most accessibility using a cloud platform."

Upcoming Features

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In February of 1977, Fredrick Sanger and his colleagues published the first sequence of an organism's complete genome, the 5,375 nucleotides of bacteriophage phiX174. Even then, it was clear that studying whole genomes would become cumbersome and tedious as scientists sequenced more complex organisms. Fortunately, the nascent field of genomics didn't have to wait long for a solution; just four months later, a small startup company in Cupertino, California began selling the Apple II to electronic hobbyists. Scientists quickly discovered that the new, relatively inexpensive computing systems were ideal for storing and analyzing gene data.

Today, it's virtually impossible to imagine molecular biology without computers. Researchers routinely search massive online databases for novel connections between genes while highly automated sequencing systems deliver terabytes of new data daily. Indeed, an entirely new scientific specialty, bioinformatics, has arisen to sort and study the growing trove of biological information.

Many institutions have built dedicated computing centers to handle the glut of data, but recently bioinformatics experts have started borrowing another strategy from the computer industry to avoid that

expense: cloud computing (or distributed computing). Instead of storing and analyzing data locally, cloud-based systems divide computationally intense work among hundreds or thousands of remote servers that are available on demand. Early adopters of cloud-based genomics had to write their own software for it, but computer scientists and service companies are now adding user-friendly interfaces to make the technique more broadly available.

THE SKY IS THE LIMIT

The most obvious argument for cloud computing is the sheer volume of new sequence data. "We're not a particularly large campus, and we have the capacity to generate about one terabyte of data per day," says Michael Schatz, assistant professor of quantitative biology at **Cold Spring Harbor Laboratory** in Cold Spring Harbor, New York. That's enough to fill the entire hard drive of a typical desktop computer in just two or three days.

Worldwide, explains Schultz, DNA sequencing machines produce about 15 petabytes of data per year (and is increasing rapidly); a petabyte is 1,000 terabytes. Writing 15 petabytes to high-capacity DVDs would produce a stack of disks about two and a half miles tall, just for the raw sequences. Experiments that include phenotypic information such as microscopy slides multiply the storage problem even further.

Fortunately, companies with deep pockets and extensive computing experience have already solved data handling problems on that scale. Google, for example, collects and processes dozens of petabytes of information about its users daily. "That's more data processed in a single day than the amount of [sequencing] data generated in the entire world in a single year," says Schatz.

To accomplish this, Google uses cloud-computing-based technology, which splits the work among a "cloud" of hundreds or thousands of servers in computing centers scattered around the world. Researchers can access a similar level of distributed computing power inexpensively and easily through services such as Amazon's EC2 system, which lets anyone rent access to a similarly huge server cloud.

Before rushing into the cloud, though, researchers should assess their needs and local resources. A computing center at a scientist's home institution can often provide faster, cheaper service than a remote cloud system for data that don't need to be shared with distant collaborators. As a rule of thumb, Schatz suggests that "if your data involves more than a hundred terabytes that needs to be shared amongst

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collaborators, I think you'll get the most accessibility using a cloud platform."

Institutions that don't have dedicated computing centers may also find the cloud attractive. "Traditionally you go and build a big data center with lots of machines in it, but that's expensive and half the time it's sitting around idle, so the benefit of cloud computing is [that] you're only paying whilst you're using the service, and the rest of the time it's no cost to you whatsoever," says Richard Holland, chief business officer at **Eagle Genomics** in Cambridge, United Kingdom.

A DIFFERENT KIND OF CLOUD ATLAS

Besides access to a huge number of remote servers, a typical cloud service also provides fundamental software. Much of the cloud computing industry now relies on free, open-source tools such as the widespread Apache server software and an add-on to Apache called Hadoop. The former handles the basic communication between each server and the network, while the latter is designed to take complex computing tasks and distribute them efficiently between thousands of servers.

Web companies originally developed this type of architecture to handle their own needs—Hadoop processes all of the world's Facebook photos and Yahoo searches—but in 2009 Schatz and his colleagues began using it for genomic data. Since then, Hadoop has become a top choice for bioinformatics in the cloud. Having "many hundreds of terabytes or petabytes of data that all need to be analyzed at once [is] becoming the de facto standard in the life sciences," says Schatz.

One major attraction of Hadoop is that it's easy to use, at least for scientists familiar with computer programming. "Just a little knowledge of Java programming is enough to be able to run large analysis tasks on very large clusters, and that's a big advantage of using Hadoop," says Jens Dittrich, a professor of information systems at **Saarland University** in Saarbrücken, Germany. Instead of having to keep track of which processor is handling which tasks, programmers can simply write their algorithms as if a single machine was doing the work, and Hadoop handles the underlying complexities of dividing the processing across thousands of servers.

Cloud-based computing in general, and Hadoop in particular, do have some drawbacks. In order to analyze data in the cloud, researchers first have to put it there, and terabyte-size uploads often take hours even over fast internet connections. Because it lacks the sophisticated indexing systems many databases use, Hadoop can also be inefficient for some types of analyses. A properly structured index allows a program to identify the specific pieces of data that are most likely to be necessary for a particular query; a system without indexes has to search the entire data set, which takes much longer.

Dittrich and his colleagues recently tackled both problems. The team's new Hadoop Aggressive Indexing System creates numerous indexes of a set of data while it's being uploaded to the cloud, using what would normally be wasted computing time to build a useful tool for optimizing subsequent analyses. Depending on the types of questions researchers are asking, the indexes can accelerate processing by a hundredfold. "It's not a silver bullet, to be fair, it depends on the analysis task ... but for many tasks we're doing pretty well," says Dittrich.

Even as new techniques make Hadoop more useful, experts in the field stress that it will never be a universal solution. Both Dittrich and



Schatz argue that cloud-based systems excel at answering some biological questions, but not others. Aligning sequencing reads, identifying gene variants, and sorting through RNA expression profiles are all good candidates for cloud-based solutions, as they require searching through large sets of data for individual pieces of information. Metabolic modeling, on the other hand, involves performing complex calculations on smaller sets of data, and may work better on a local computing system.

BIG DATA FOR THE REST OF US

Hadoop also doesn't make sense for biologists who aren't comfortable writing their own computer programs. For those scientists, several companies now offer user-friendly interfaces for cloud-based data analysis.

"The cloud comes in a variety of different sorts of flavors," says Eagle's Holland. Options range from bare-bones server leasing arrangements, often called infrastructure as a service, to fully built applications or software as a service (SaaS).

With SaaS, a service company provides the cloud infrastructure, data storage, and bioinformatics software. In many cases, researchers can have their sequencing data sent directly to the company, then perform common types of analyses in a point-and-click web environment. Sequencing companies such as Illumina in San Diego, California now offer their own SaaS systems, while numerous startup companies are also exploring this new niche.

Each service company has its own approach. Eagle Genomics, for example, links different prebuilt programs together to tailor software for each user. "People usually come to us and say 'we need to build an analysis pipeline that will do SNP prediction or variant calls,'" says Holland, adding that the company will then take published algorithms and "plumb them together into a ... workflow that answers their question." Researchers can then use the customized workflow to analyze their data on a cloud of servers. More experienced users can also delve into the computer code themselves to modify it.

Investigators looking for an even easier entry into the cloud can turn to one of the many companies now offering general-

"A good way of doing a backup is to have a write-once medium, a [non-rewritable] DVD is a good example, you burn it once and you can't overwrite it anymore."

Featured Participants

Cold Spring Harbor Laboratory
www.cshl.edu

DNA Nexus
dnanexus.com

Eagle Genomics
eaglegenomics.com

Spiral Genetics
spiralgenetics.com

Saarland University
www.uni-saarland.de/en

Additional Resources

Amazon EC2
aws.amazon.com/ec2

Hadoop
hadoop.apache.org

Illumina
www.illumina.com/
software/basespace.
ilmn

purpose software that address common types of queries. “There’s a lot of functionality that a biologist can access in our service just by logging in and clicking buttons in their web browser,” says Andreas Sundquist, chief executive officer and co-founder of SaaS provider **DNA Nexus** in Mountain View, California.

Though SaaS companies often develop their own proprietary code and interfaces, scientists shopping for cloud services should still ask about the underlying algorithms. “Researchers are a conservative bunch of people really, they like algorithms that have been published and tested and peer reviewed and well understood, and are less keen to experiment with new techniques on important data,” says Holland.

Fortunately, most of the new bioinformatics companies are happy to discuss their systems. “All of the algorithms that are currently integrated into Spiral are peer reviewed, [and] we definitely get that people want to use things that are open source,” says Adina Mangubat, chief executive officer of **Spiral Genetics** in Seattle, Washington. Spiral puts its own interface and data-handling layer on the published algorithms to make them easy to use. Other companies in the field echoed that sentiment, and most SaaS leases allow researchers to access the underlying software code directly.

CLOUD COVER

Because cloud computing is relatively new, researchers in some fields remain skeptical of it. That’s particularly true for pharmaceutical and biomedical scientists who handle sensitive proprietary data or information about patients. “There’s definitely a perception of being able to control what’s going on in your local cluster more than being able to control what’s happening in a cloud environment,” says Mangubat.

That concern may not be well founded. Studies have shown that three quarters of recent medical security breaches in the United States have been the result of clinicians losing laptop computers or portable storage drives. “If they had used the cloud instead ... stealing a laptop would’ve been a non-issue, because you would’ve never had the patients’ data on that laptop to begin with,” says Sundquist.

Indeed, as banking, government, and e-commerce companies have

moved their data into cloud storage, security at server facilities has become extremely robust. Companies targeting the medical research market have also paid close attention to data security laws. “One of our fundamental tenets is making sure we have enterprise-grade security and all the features necessary to operate in a clinical or diagnostic setting,” says Sundquist.

Even scientists renting bare cloud infrastructure and writing their own algorithms should expect strong security. Mangubat points out that the popular Amazon EC2 cloud leasing service already complies with regulations for the physical security of medical data, leaving only the researchers’ own software as a potential weak point.

FOGGY MEMORIES

Another common concern with cloud computing—and something researchers should ask about before signing a server lease—is archiving. If a SaaS company shuts down or a researcher decides to switch to a different system, the lease should specify a way to retrieve the data. “We offer services that will allow us to burn all of that stuff to disk and ship them a big stack of hard drives, you’re not married to the cloud for life,” says Mangubat.

For general storage, though, the cloud can offer protection from accidents and local disasters, as cloud services typically replicate data across multiple locations. “You could have a meteor hit one of the data centers and a volcano erupt in the other one, but you’ll still have another copy of your data,” Sundquist explains.

Cloud storage could also help address the tricky problem of archiving digital information. For example, data stored on standard computer floppy disks just a few decades ago are often unreadable today, as the now-obsolete disk drives and operating systems are no longer available. In cloud-based storage, workers constantly transfer data to new media, while version-control systems preserve old editions of the software. Future researchers should be able to resurrect both the data and the tools used to analyze them.

Not everyone is satisfied with that solution, though. “As long as it can be overwritten, it’s not an archive,” says Dittrich. To prevent valuable sequencing data from being devoured by a computer bug or human error, he recommends storing an extra copy on another type of media. “A good way of doing a backup is to have a write-once medium, a [non-rewritable] DVD is a good example, you burn it once and you can’t overwrite it anymore,” he says.

As the petabytes continue piling up, though, some experts suggest that the ultimate archiving system for genomic data might be DNA itself, completing the connection between computing and biology. In this view, it may soon be cheaper and faster to resequence a stored biological sample than to retrieve the original sequence data from a digital archive. “Today there is a several day lag and expense to sequencing DNA, but it’s kind of a sneak peek into the future ... where if sequencing was really more or less instantaneous, it does have some merit to being an information storage medium,” says Schatz.

Alan Dove is a science writer and editor based in Massachusetts.

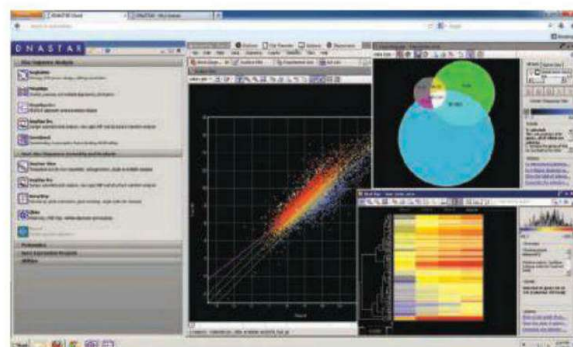
DOI: 10.1126/science.opms.p1300077

NEXT GENERATION SEQUENCE ANALYSIS

Lasergene—an integrated suite of software for Sanger and next generation sequence assembly and analysis—is now available on the Amazon Cloud with version 11. By using the cloud, researchers can more easily collaborate globally, access powerful hardware for occasional large projects that require it, run as many assembly projects as desired sequentially or concurrently, and take full advantage of the flexibility offered by our software for any application anywhere, anytime. Other significant improvements with Lasergene 11 include the introduction of a new application, MegAlign Pro, which includes the Muscle sequence alignment algorithm; new 16S rRNA and host-viral integration workflows in support of next generation sequencing platforms and technologies; enhanced Copy Number Variation analysis capability; and numerous improvements to Protean 3D, the integrated protein structure, sequence, and bioinformatic application within the Lasergene software suite.

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EPIGENETIC ANALYSIS SERVICES

Services are now available for investigating DNA methylation (5-mC) and hydroxymethylation (5-hmC) as well as targeted DNA methylation analysis of single or multiple genetic loci. Multiple service options are available to researchers, each offering varying degrees of genome coverage. No experience with sequencing or bioinformatics technologies is required—simply submit the samples and receive high-quality, easy-to-interpret publication-ready data and figures. There are many unique benefits to using the services, including the most highly cited chemistries for bisulfite treatment of DNA for 5-mC analysis, novel library prep workflows for ultralow DNA inputs, and custom-designed bioinformatics pipelines to ensure seamless data handling, analysis, and delivery. Additionally, a novel technique is available to determine DNA hydroxymethylation levels on a genome-wide scale called Reduced Representation Hydroxymethylation Profiling (RRHP), which is the only method available for reliable single base-pair resolution and strand-specific profiling of 5-hmC modifications in DNA.

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The **US \$1 million** prize will be awarded for global innovation or a major scientific or technological breakthrough in the field of alternative fuels for transportation.

Nominees for the prize should be actively engaged in innovative, paradigm-shifting research in the field of alternative fuels for transportation, worthy of significant and widespread attention. The recipient(s) can be of any nationality, race, ethnicity, or sex.

Deadline for nominations: Extended until June 30th, 2013

For nomination information and forms see
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Research Microbiologist (Immunology) or Research Veterinary Medical Officer GS-12/13

Salary Range of \$68,809 to \$106,369

The Food Safety and Enteric Pathogens Research Unit at the National Animal Diseases Center in Ames, Iowa, is seeking a permanent full-time Research Microbiologist (Immunology) or a Research Veterinary Medical Officer to conduct research on the interactions between intestinal commensal microbes, notably food-borne pathogen *Campylobacter* species, and mucosal defense mechanisms of turkeys.

The scientist will use traditional, genomic, and transcriptomic immunological techniques to describe the development of turkey intestinal mucosal defenses after hatch and in response to different environmental conditions; will perform individual and collaborative team research to design and evaluate food safety control strategies for *Campylobacter* in turkeys, such as prebiotic and innate immunological approaches; will evaluate immune responses and efficacy following exposure to *Campylobacter* immunogens. The research has the goal of identifying non-antibiotic strategies to reduce *Campylobacter* colonization of poultry on the farm, the first link in the human food chain. Team members and collaborators include microbiologists, molecular biologists, pathologists, veterinarians, and animal scientists.

NADC is the premier research institute within the USDA for studying diseases of large animals and has recently undergone a \$500 million upgrade of laboratory facilities. NADC resources for the position include flow cytometry, mass spectroscopy, laser capture microdissection microscopy, a nucleic acid center operating Roche FLX, Illumina, MiSeq, AP3100 sequencers, an electron microscopy/histology facility, bioinformatics resources, and both conventional and gnotobiotic large animal support. NADC researchers also have access to resources and collaborators at nearby Iowa State University. At the NADC, scientists are able to investigate microbe-host interactions at every level—molecular, microbe, and natural host.

Beginning **June 24, 2013**, details of this position, including salary, qualification requirements and application directions will be described in Job Announcement ARS-X13E-0092 posted on the USAJOBS website, www.usajobs.gov. **U.S. citizenship is required.** Applications can be faxed, e-mailed or mailed (post-marked) until **August 16, 2013**.

Contacts: Application procedure: Kim Grandon, Kim.Grandon@ars.usda.gov; 515-337-7277
Scientific information: Thad Stanton, Thad.Stanton@ars.usda.gov; 515-337-7350

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Wayne State University (WSU) is seeking to strengthen its Placental Pathology Unit, whose goal is to characterize the frequency and clinical significance of human placental lesions, and to study the mechanisms of disease whereby these lesions arise and have consequences in fetal and maternal health and disease.

The Placental Pathology Unit collaborates with Departments within the School of Medicine, and also with the Perinatology Research Branch of the Division of Intramural Research, NICHD, NIH, DHHS, which is housed at the WSU campus.

This unit applies techniques of molecular pathology to the study of normal and abnormal placentation. The successful applicant should have an M.D. degree or M.D./Ph.D., have completed a residency training in surgical pathology, and have demonstrated experience with the nosology of placental lesions.

Experience and expertise in the methods of placental sampling, gross and histopathological examination, immunohistochemistry, in-situ hybridization, and immunofluorescence are required. Familiarity with state-of-the-art methods for optical imaging (such as confocal and intravital microscopy) is desirable. The successful applicant is expected to establish a program of molecular pathology of the placenta and an experimental component focused on the biology of trophoblast and fetal membranes in normal pregnancy, as well as in parturition.

The primary focus is the conduct of original research with a strong interdisciplinary component. The team includes maternal-fetal medicine specialists, neonatologists, geneticists, other pathologists, scientists, as well as residents and fellows. The applicant is expected to lead a clinical pathological conference, and teach surgical pathology of the placenta to residents, fellows, and other members of the research team.

Impeccable written and oral communication skills are required to disseminate results of research at scientific meetings and to write scientific papers. The applicant will supervise laboratory staff which provides support for gross and surgical pathology.

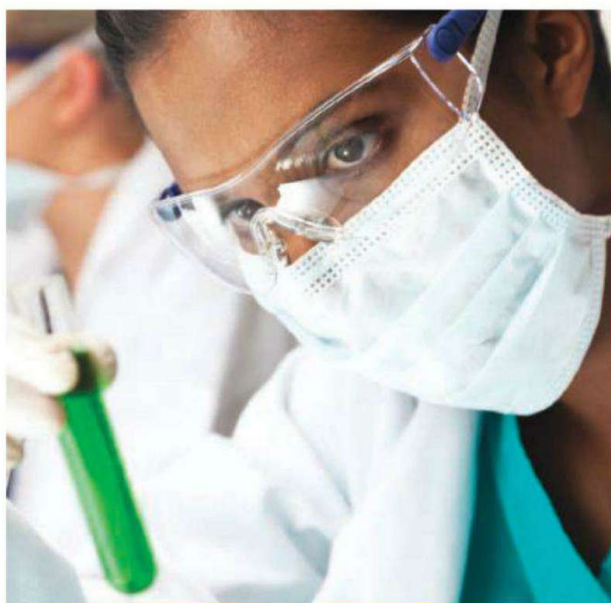
The Placental Pathology Unit offers an exceptional opportunity for academic growth for individuals committed to the pursuit of excellence. Applications from the U.S and non-U.S. graduates are welcome. Tenure and non-tenure track positions are available.

Review of applications will begin immediately, and will continue until the position is filled.

Interested individuals should send:

- a curriculum vitae,
- a separate statement summarizing their experience and professional contributions,
- and a list of three references to:

Sonia S. Hassan, M.D.
Associate Dean for Maternal, Perinatal and Child Health
Wayne State University School of Medicine
mpch@med.wayne.edu



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Division of Research and Economic Development Position of Director Biomanufacturing Research Institute and Technology Enterprise

The Division of Research and Economic Development is seeking a Director, Biomanufacturing Research Institute and Technology Enterprise (BRITE) to provide the overall leadership and supervision of the unit. BRITE is one of NCCU's major research institutes and house the Department of Pharmaceutical Sciences. The incumbent is expected to have an earned Ph.D. (or M.D. or PharmD) in Biochemistry, Chemistry or Biology or a related field with a minimum of 10 years of experience in the pharmaceutical industry. It is expected that the incumbent will qualify for full professor and tenure. The Director reports to the Vice Chancellor for Research and Economic Development.

Research within BRITE is designed to address biomanufacturing and drug discovery. The Institute boost of its 460,000 compound library which is the largest at any college or university in the United States of America. BRITE serves approximately 200 students, annually, who are majoring in pharmaceutical sciences at the undergraduate and/or graduate levels. The newest graduate program within the unit is the Ph.D. Integrated Biosciences/Pharmaceutical Sciences Track. BRITE serves as the major research arm of the Department of Pharmaceutical Sciences, College of Arts and Sciences. The unit has approximately 38 research/faculty and staff members.

The Division anticipates filling the Position of Director, Biomanufacturing Research Institute and Technology Enterprise by **September 3, 2013**; however, review of applications will commence immediately and continue until the position is filled. Applications and/or Nominations may be submitted to the person whose name and contact information is listed below: **Hazell Reed, Ph.D., Vice Chancellor, Division of Research and Economic Development, Suite 309 Hubbard-Totton Building, 1801 Fayetteville Street, North Carolina Central University, Durham, NC 27707**; or via email hreed@nccu.edu.

THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

School of Science Joint Faculty Positions

The School of Science of The Hong Kong University of Science and Technology seeks applicants for joint tenure-track positions at the rank of Assistant Professor or Associate Professor. The School is seeking applicants with expertise in interdisciplinary areas, such as super-resolution imaging and biological physics, that bridge life science, physics, and chemistry. Successful applicants should have a doctoral degree plus several years of postdoctoral experience. They will be expected to establish an independent, internationally recognized research program and to contribute to the undergraduate and graduate teaching missions of the School.

The School of Science is located in the vibrant international atmosphere of the University, on a quiet, picturesque sea-side campus, just 40 minutes from downtown Hong Kong. Teaching and research are carried out in an outstanding intellectual environment that is rich in technical resources. The medium of instruction in the University is English.

Starting salary will be commensurate with qualifications and experience. Fringe benefits including medical/dental benefits and annual leave will be provided. Housing benefits will also be provided where applicable. The School of Science is committed to diversity in its ranks and strongly supports equal opportunity employment.

Applications should include a curriculum vitae, a short statement of research interests and the names and addresses of three individuals who can serve as referees for the candidate. These materials should be sent to Prof. Yung Hou Wong, Chair of the **Adhoc Joint Search and Appointment Committee for Interdisciplinary Recruitment, Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong**. Electronic submissions are strongly encouraged (email: indissearch@ust.hk). Review of applications will start immediately and will continue until the positions are filled.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)

Wayne State University (WSU) seeks nominations and applications for a full-time faculty position focused on the study of microorganisms in pregnancy complications. The programmatic goal is to establish an exceptional unit to characterize the microbiome of the genital tract and discover pathogens in normal pregnant women and those with complications. A priority is to develop molecular techniques for the identification of bacteria and viruses responsible for intra-amniotic and congenital infections using sequence-based techniques.

A molecular microbiology unit, specific to reproduction, will be established to strengthen the University's impressive record of innovative discoveries and achievements in obstetrics, maternal-fetal medicine and perinatal medicine. The unit is intended to promote collaboration among clinicians and faculty members working in reproductive immunology, genomics and computational biology. This position is part of the WSU Perinatal Initiative to create partnerships with the Perinatology Research Branch of the Division of Intramural Research, NICHD, NIH, DHHS, housed at the WSU campus.

The successful candidate is expected to establish a productive and independent research program in the area of molecular microbiology. A Ph.D. degree or equivalent, expertise and training in the identification of microbes (bacteria and viruses) using sequence-based techniques are requirements. The program is intended to combine morphologic (in situ hybridization), culture, and sequence-based techniques to the characterization of infections during pregnancy (maternal and fetal) and to study changes in the microbial ecosystem. Emphasis will be on the rapid identification of pathogens, microbial sequencing, transcriptomics and visualization of bacteria in tissues. The faculty member should be able to establish a new laboratory, participate in graduate and medical education, recruit and supervise laboratory staff, and lead a productive and dynamic team.

WSU is committed to academic excellence and diversity within the faculty, staff and student body. WSU is interested in candidates who have demonstrated commitment to excellence in research and teaching. Some scholarly activity and service towards building an equitable and diverse scholarly environment is required. Successful candidates should possess excellent written and verbal communication skills. Salary is commensurate with qualifications and experience and based on the WSU pay scale. Tenure and non-tenure track positions are available. Series of appointment, as well as a competitive start-up package, will be determined based upon the candidate's skills, qualifications and experience. National and international applicants are welcome.

Review of applications will begin immediately, and will continue until the position is filled.

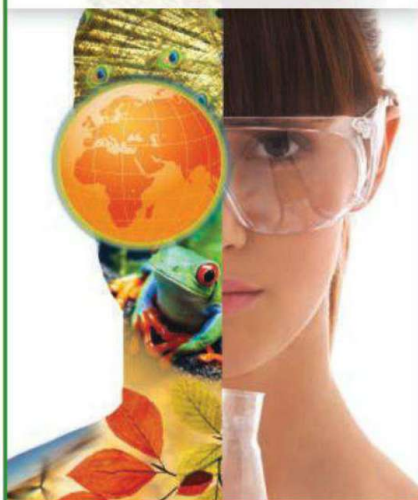
Interested individuals should send:

- a curriculum vitae,
- a separate statement summarizing their experience and professional contributions,
- and a list of three references to:

Sonia S. Hassan, M.D.
Associate Dean for Maternal, Perinatal and Child Health
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School of Medicine & Health Sciences

THE GEORGE WASHINGTON UNIVERSITY

SENIOR ASSOCIATE DEAN FOR RESEARCH

The George Washington University (GWU) School of Medicine and Health Sciences (SMHS) invites applications and nominations for the Senior Associate Dean for Research. The individual appointed to this position will report to the Vice President for Health Affairs and Dean. Primary responsibilities will include supporting and enabling world-class scientists to procure research funding, assess and identify areas for research expansion based on existing areas of research excellence and funding opportunities, cultivating research relationships with outside entities, leading research-related faculty development and developing a plan to secure the infrastructure, funding and technologies required to advance our research mission.

GWU and Children's National Medical Center are partners in research and academics, and share a prestigious Clinical and Translational Science Award (CTSA) from the NIH. In addition, the SMHS is currently undergoing a major \$15 million NIH-funded CO6 laboratory renovation. The SMHS research capacity will be further increased with the completion of a new 500,000 square foot Science and Engineering Hall that will include significant space for medical research. Areas of special research expertise and emphasis include, but are not limited to, the study of neurological disorders, cancer, HIV/AIDS, neglected infections of poverty, and cardiovascular disease.

Basic Qualifications: Applicants must have a M.D. and/or Ph.D. degree with an outstanding record of funded research, experience in research or scientific administration, and be skilled in working collaboratively in a complex environment. Salary is commensurate with experience and appointment may be tenure eligible at the Associate or Full Professor level.

Application Procedure: We invite application from all qualified individuals, including those from academic medical centers and industry. Nominees and applicants should submit their CV and a bullet point summary of their research accomplishments electronically to **search committee chair, Dr. David Mendelowitz and to our search consultant, Dr. Ilene H. Nagel at GWU.Research@russellreynolds.com**. Application review will begin on **July 12, 2013** and continue until the position is filled. Interested parties should submit their materials as soon as possible. Only complete applications will be considered.

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Wayne State University (WSU) is seeking to establish a Unit devoted to the study of epigenetics in pregnancy and pregnancy complications.

The goal of the Unit is to study the effect of environmental factors in inducing epigenetic changes (DNA methylation, histone modification, microRNA, etc.). The first step would be to catalog epigenetic modifications taking into account tissue and cellular specificity. The study of the effect of exposure to microbial products and microorganisms during fetal life in the immune system (adaptive and innate) epigenome is a priority. The Unit is expected to use the tools of epidemiology to identify associations and to move to experimental studies addressing the issues of causation using in-vitro and in-vivo experimentation.

The Epigenetics in Pregnancy and Fetal Development Unit collaborates with Departments within the School of Medicine, and also with the Perinatology Research Branch of the Division of Intramural Research, NICHD, NIH, DHHS, which is housed at the WSU campus.

The successful applicant should have a Ph.D. degree, and be able to establish a dynamic program whose primary focus is original research. After 3 years, it is expected that external funding can be secured.

The primary focus is the conduct of original research with a strong interdisciplinary component. The team includes maternal-fetal medicine specialists, neonatologists, experts in genomics, computational biologists, pathologists, reproductive biologists, as well as residents and fellows. The applicant is expected to lead a scientific group, organize regular seminars and Journal clubs to participate in education/training, and critical analysis of progress in the field. Impeccable written and oral communication skills are required to disseminate results of research at scientific meetings and to write scientific papers.

The Epigenetics in Pregnancy and Fetal Development Unit offers an exceptional opportunity for academic growth for individuals committed to the pursuit of excellence. Applications from the U.S and non-U.S. graduates are welcome. Tenure and non-tenure track positions are available.

Review of applications will begin immediately, and will continue until the position is filled.

Interested individuals should send:

- a curriculum vitae,
- a separate statement summarizing their experience and professional contributions,
- and a list of three references to:

Sonia S. Hassan, M.D.
Associate Dean for Maternal, Perinatal and Child Health
Wayne State University School of Medicine
mpch@med.wayne.edu

There's only one

DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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XJTU-HKUST
JOINT SCHOOL OF SUSTAINABLE DEVELOPMENT
under planning



西安交通大学
XI'AN JIAOTONG UNIVERSITY



香港科技大學
THE HONG KONG
UNIVERSITY OF SCIENCE
AND TECHNOLOGY

Founding Department Heads

The Hong Kong University of Science and Technology (HKUST) seeks established academics with a vision to advance education and research in sustainable development to help build in partnership with Xi'an Jiaotong University (XJTU) the planned XJTU-HKUST **Joint School of Sustainable Development** (JSSD), a strategic alliance to combine the strengths of the two universities to address needs on both the regional and global level. To be located in Xi'an, the gateway to the fast-emerging western region of China, the School will focus on training a new generation of graduates who are capable of contributing to sustainable development, a strategic national goal for China and an area that is widely recognized as one of the world's most pressing challenges. English will be the School's language of instruction.

HKUST is a world-class leading research university and has been ranked overall No. 1 university in Asia for the last two years by QS Asian University Rankings®. XJTU, established in 1896, is in the C9 League, which consists of the top nine prestigious universities in China. Both universities are internationally known for their research and educational programs, especially in the areas of the natural and social sciences and engineering. By combining the strengths of the two universities, JSSD will make a fundamental contribution to education in the field of sustainable development and undertake interdisciplinary high-impact research in energy conservation, resource management and environmental protection. Within the next five years, the School is expected to have up to 60 faculty members and an enrollment of more than 1,000 undergraduate and postgraduate students in three departments:

The **DEPARTMENT OF SUSTAINABLE ENERGY** is an interdisciplinary academic unit to be established to provide undergraduate and postgraduate academic programs and undertake cutting-edge research in energy-science and technologies for sustainable development focusing on sustainable use of fossil fuels and their environmental impact, alternative energy sources, energy distribution and storage, system and engineering design for sustainability, and energy policy and management.

The **DEPARTMENT OF SUSTAINABLE MATERIALS** will provide a broad-based undergraduate and postgraduate education in materials science and materials development for sustainability, targeting the frontiers of materials science, and will undertake cutting-edge research in materials sustainability. The academic foci are: fundamentals of materials science and engineering for sustainability; sustainable adoption of materials; design of new materials; and application of sustainable materials.

The **DEPARTMENT OF SUSTAINABLE INFRASTRUCTURE** will provide education and research platforms to study interrelationships among economic and urban development, governance, and regional, national and global ecosystems focusing on the social and physical infrastructure needed for sustainability. The academic foci are: system analysis and related infrastructural issues; public policy and sustainable development; climate dynamics and environmental policy; sustainable living environments; and commerce and ecosystem management.

The founding Department Heads of the three departments will be senior faculty members of HKUST appointed for posting to the JSSD in Xi'an. We are now seeking world-class scholars in the research areas of energy, materials or infrastructure to fill these senior faculty positions at HKUST with tenure. Applicants must meet the high academic and professional standards of the HKUST senior faculty. Candidates appointed to the founding headships, reporting to the Dean of JSSD, will lead the formation of the departments, formulate governance policies, facilitate academic advancement in teaching and research, and take charge of faculty recruitment and resource allocation. They will typically have substantial experience and proven leadership skills in managing major research programs and human resources in an academic or large-scale research setting. They will also be able to help promote the department at the global level, develop collaborative programs and attract major external funding for large interdisciplinary research projects at the national level. We are also ready to explore other opportunities with scholars interested in teaching and research in the relevant areas.

Remuneration is highly competitive and will be commensurate with qualifications and experience. Generous fringe benefits, including housing where appropriate, will also be provided.

Nominations and applications, including a curriculum vitae, a vision statement of the development of the new departments, a brief statement of current interests, as well as the names, addresses, phone numbers and email addresses of at least three references should be sent to: **The JSSD Search Committee, c/o Human Resources Office, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong**, or by email to: jssdsearch@ust.hk. Review of nominations/applications will begin immediately and will continue until the positions are filled.

For further information about HKUST and XJTU, please visit the following websites:

HKUST - <http://www.ust.hk>

XJTU - <http://www.xjtu.edu.cn/en/index.html>

(Information provided by applicants will be used for recruitment and other employment-related purposes only.)



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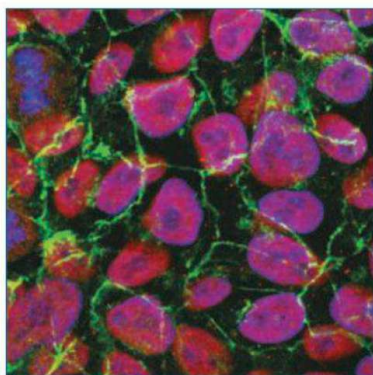




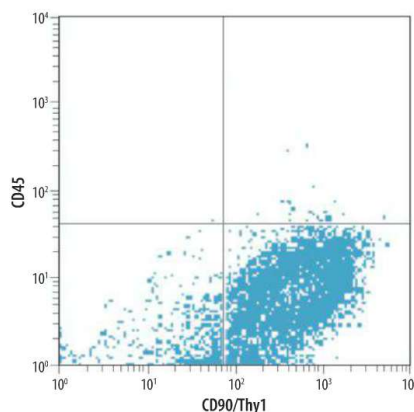
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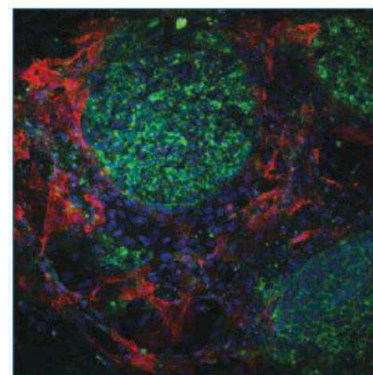
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